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THE PHYSIOLOGY
OF
INDUCED HYPOTHERMIA

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Robert D. Dripps, M.D.
Chairman and Editor



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This Symposium was undertaken by the National Academy of Sciences—National Research Council as a result of discussions with the Research and Development Division, Office of the Surgeon General, U. S. Army. The costs of the meeting were defrayed jointly by the Army, Navy and Air Force, and the Army contributed additional funds to support publication of these Proceedings.

The Symposium was organized and conducted under the auspices of four subcommittees of the Committee on Medicine and Surgery, in the Academy-Research Council's Division of Medical Sciences—the Subcommittee on Anesthesia, the Subcommittee on the Cardiovascular System, the Subcommittee on Shock, and the Subcommittee on Trauma.

Dr. R. D. Dripps served as Chairman of a planning conference on 21 March 1955, as well as of the Symposium, and also as editor-in-chief of these Proceedings. Lt. Col. W. H. Crosby, Jr., and Drs. M. E. De Bakey, A. H. Hegnauer, F. J. Lewis, F. D. Moore, and W. H. Muller, Jr., presided at various sessions of the Symposium. In the Division of Medical Sciences, staff responsibility was borne chiefly by Lt. Cdr. C. D. West, who organized the Symposium under the guidance of Dr. M. H. Sloan and Dr. Thomas Bradley, and by Dr. D. E. Copeland, who assembled and prepared the manuscript for Dr. Dripps. Mr. Frank M. Holz was retained to complete the final editing of the Proceedings and prepare them for publication.

Some regrouping of the papers was found desirable. The discussions have also been rearranged and much condensed. A brief report by Capt. T. G. Barila concerning work then in progress was later withdrawn at his own request. Since the work of Drs. R. K. Andjus, J. E. Lovelock, and A. U. Smith was presented chiefly by means of a motion picture, a summary of the film and relevant addenda were submitted after the Symposium. The five articles of Review and Appraisal were also prepared subsequent to the meeting.

FOREWORD

PHILIP S. OWEN

In his introduction the Chairman, Dr. Dripps, points out how this conference arose from small beginnings. We thought originally that interest in this subject would be rather limited and specialized. It has proved otherwise, and we owe a measure of apology to many whom we had, of necessity, to turn away.

On behalf of the Division of Medical Sciences of the National Academy of Sciences—National Research Council I want to express our appreciation to Dr. Dripps and to the members of the several committees who worked long and hard in the preparation of this conference, and to the participants who bore with us in these preparations. They are too numerous to thank individually here. I want especially to thank our guests who came from abroad—from Britain, Sweden, France, Holland, and Yugoslavia. I hope they found their journey rewarding.

Finally, we are most grateful to the Armed Forces who together, not only through their interest in the practical application of cold to problems of medical practice but also through their concern with the fundamental underlying physiological mechanisms, have sponsored and made possible this meeting. The three services have contributed jointly to the support of the Symposium, but particular credit is to be given to the Army for giving initial impetus to the program and for its generous support of this publication.

INTRODUCTION

ROBERT D. DRIPPS

The interest of the Armed Forces in hypothermia was aroused by the possibility of using lowered body temperature to prevent the onset of shock, to treat shock once it had developed, and to permit surgical intervention on patients who might be unable to tolerate the stress of anesthesia and operations at normal body temperature. The initial interest in hypothermia was therefore primarily of a practical nature.

As clinicians began the practice of reducing the body temperature of patients, it became evident that much additional information was needed on the fundamental alterations in function which accompanied hypothermia. Until these physiologic factors were understood, the use of hypothermia would remain largely empirical. Furthermore, certain primary hazards associated with the lowering of body temperature were soon recognized. Rather than approach these in the clinic by trial and error, it seemed essential to enlist the aid of individuals with a basic orientation in various of the medical sciences.

This conference was consequently designed to bring together clinicians with a practical experience in the problems of induced human hypothermia and research workers with a broad background of related interests. It was our hope that the more important questions in hypothermia would be delineated, that profitable areas of investigation would be outlined, and that through the stimulus of the conference some would return to the laboratory for a fresh look at this intriguing subject.

The large number of formal presentations and the breadth of the subject limited the time available for free discussion, appraisal, and synthesis of the material. In consequence, several of the participants were asked to review the data presented and to offer for inclusion in these pages an evaluation of specific sections. It was hoped that the reviewers would call attention to conflicting data and opposing theories; that the sources of such differences would be pointed out in terms of such variables as differences in technique, experimental design, species studied, temperature range, and degree and type of anesthesia; and that directions for future work would be indicated. This has been attempted by J. W. Severinghaus and S. M. Horvath for the general physiological aspects of hypothermia; by C. McC. Brooks and B. F. Hoffman for cardiac irritability; and by R. D. Dripps for the clinical and technical phases of induced hypothermia in man. These will be found at the end of the particular sections concerned.

I should like to take this opportunity to acknowledge the tremendous amount of effort expended by Drs. Charles D. West and D. Eugene Copeland of the staff of the Division of Medical Sciences of the Academy-Research Council in the preparation of this conference. Their help has been invaluable.



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PART I

SOME CONSIDERATIONS OF PHYSICO-CHEMICAL FACTORS IN HYPOTHERMIA

DUGALD E. S. BROWN

A consideration of physicochemical factors in hypothermia is a complex assignment. The concept of homeostasis, so well developed by the late Prof. Cannon and stemming from Claude Bernard's vision of the milieu interior, is sufficient to give anyone a conservative view on the direct role of physical chemistry in hypothermia. I am honored at being given this opportunity to open problems for consideration, but I am approaching this assignment chiefly as a physiologist with a strong interest in physicochemical biology.

The regulation of intra-animal affairs and the maintenance of homeostasis, in the final analysis, rests at the cellular level. If the cardiac output and the chemical composition of the blood are sufficient to meet the cellular requirements, the regulatory mechanisms will remain effective and the survival of the animal will be assured. On the other hand, when the rate of oxygen utilization exceeds the rate of oxygen transport, cellular activities are reduced and regulation is impaired.

In induced hypothermia, the low temperature slows the rates of all processes and modifies the action of metabolites and other substances. This in itself is not necessarily harmful, as shown by the true hibernator, but will become disastrous as soon as anoxia and chemical imbalance begin to develop. The excellent experiments of Gollan *et al.* (1955) where dogs, provided with an adequate composition and circulation of blood by artificial means, survived cooling to 1.5° C., point directly to circulatory failure as the limiting condition in hypothermia with anoxia and chemical imbalance as primary agents modifying the activity of the regulatory cells.

The basic physicochemical considerations in hypothermia thus relate to the laws governing the dependence of cellular activities and their enzymatic reactions on temperature, ions, metabolites and drugs. Of particular importance are such cellular phenomena as excitability, rhythmicity and contractility. In regulating the oxygen transport these can act interdependently, since their specific rates are set at complementary levels. When the temperature is lowered, the rates are reduced in accordance with the temperature coefficients of the respective processes. In such an interdependent system its effectiveness at any temperature depends on the actual relative rates of the processes and on their temperature coefficients.

In both the true hibernator and the non-hibernator there is every reason to expect comparable values for the temperature coefficients of the cellular reactions. For several hundred processes, including rates of diffusion, cardiac rhythms, and numerous enzyme reactions, the Arrhenius U_c values range from U_c 3,000 to 25,000 with a large number of processes grouped at U_c 6,000, 12,000 and 16,000 (Morales, 1947).

In terms of the temperature coefficient, or Q_{10} , these are grouped at Q_{10} 1.0, 2 and 3, thus indicating that the rates of the reactions involved would increase in this proportion for a rise in temperature of 10° C. or, in relation to hypothermia, a

decrease in the same proportion. In general, the rates of metabolic and rhythmical processes exhibit a Q_{10} of 3, the rates of contraction a Q_{10} of 2, and the rates of most physical processes such as diffusion, a Q_{10} of 1. As a result, when the temperature is lowered the rates of metabolic and rhythmical processes decrease two to three times as much as the rate of diffusion of the metabolites.

In both the hibernator and non-hibernator it would be expected that the temperature coefficients for identical processes would be the same. The factor contributory to the ready survival of the hibernator would be that the reaction rates of the various cellular processes have a better relative setting at 38° C. Thus on cooling, although the rates decrease, the relative values are sufficient for the over-all effectiveness of the system.

In the non-hibernator it appears that the rate setting of the processes is quite different. The end result is that, although the Q_{10} 's are the same, a lowering of the temperature reduces the rates of certain reactions to a level where they can no longer contribute effectively as members of an interdependent reaction system.

Considering that such a system is acting on events within cells, it could lead, for example, to cessation of the cardiac rhythm at 13° C. in the dog, whereas the beat of the hamster could continue at a much lower temperature. At the systemic level, acting between the heart and the nervous system, it could interfere with the nervous regulation of the heart.

In accordance with the foregoing, the control of induced hypothermia would rest on the extent to which the rates of cellular processes and their temperature coefficients could be controlled. The extent to which this could be accomplished naturally depends on a sound understanding of the cellular phenomena involved and the laws governing their susceptibility to temperature, ions and drugs.

The most significant physicochemical development bearing upon intracellular enzymes stems from the studies of F. H. Johnson and co-workers* on bacterial luminescence. In an extensive investigation of the luminescence reaction *in vivo*, its dependence on temperature, pressure and various chemical agents was established and the kinetics described in terms of the Glasstone-Eyring theory of absolute reaction rates. Recently the essential enzymatic proteins were isolated from the bacterium *Achromobacter fischeri* and light emission found to occur in the presence of FMN (flavin mononucleotide), reduced DPN (dihydrodiphosphopyridine nucleotide), and palmitic aldehyde. When this system was studied in relation to temperature, pressure and inhibitors, it was found to behave similarly to the system *in vivo* (Strehler and Johnson, 1954).

For many years it has been the hope of both physiologists and physical chemists that a specific cellular process, enzymatically controlled, could be duplicated by the isolated enzymatic proteins *in vitro*. It seems that this is being approximated in bacterial luminescence. Assurance is thus given that the physicochemical analyses of intracellular reactions can provide valuable information on the properties of the underlying enzymatic reactions which control the wide spectrum of cellular reactions. In relation to the regulation of hypothermia and its control, there is thus a good

* An extensive treatment of the physical chemistry of enzymatic and cellular processes in relation to temperature, pressure and chemical agents will be found in F. H. Johnson, Henry Eyring and M. J. Polissar: *The Kinetic Basis of Molecular Biology*, John Wiley and Sons, Inc., New York, 1954.

reason to consider the luminescence reaction as the prototype of many groups of intracellular processes, particularly in relation to temperature and chemical agents.

The temperature relations of the luminescence reaction are typical of numerous biological processes. With increasing temperature, the rate of the reaction increases in accordance with the Arrhenius equation, reaches a maximum, and then decreases (Brown, Johnson, Marsland, 1942). At low temperatures, the rate is determined by the luminescence reaction with $U. 16,000$, while at high temperatures the rapid decrease in rate is controlled by the reversible thermal denaturation (RTD) of the enzymatic proteins. The rate at intermediate temperatures then depends on the interplay between these opposing reactions. Concerning the RTD, a sufficient body of evidence has accumulated to consider that it depends on a reconfiguration of the protein enzymes, this being attended by a large increase in volume (ΔV 80–100 cc.) and a heat equilibrium (H 50,000–80,000 cal.).

The recognition that this typical temperature relation involves at least two quite distinct reactions has opened the way to an understanding of the action of various agents on cellular processes and enzymatic reactions *in vitro*. As a result of a most extensive study of the action of inhibitors, F. H. Johnson and co-workers concluded that in the simplest cases these agents fall into two classes, designated as Type I and Type II. Among the Type I compounds are agents such as sulphanilamide and certain anticholinesterases. These, it seems, combine with the prosthetic group of the enzymes and, since the degree of association is temperature-dependent, they become more effective at lower temperatures. In view of their mode of action, the Type I compounds tend to compete with the substrate and are thus influenced by variations in the effective substrate concentration.

The Type II compounds, which include a large number of narcotics, encourage the RTD and are thus greatly potentiated by a rise in temperature. Certain agents, such as quinine, exhibit both Type I and Type II effects, indicating in all probability that they are acting at more than one locus. The effectiveness of such agents is at a minimum at intermediate temperatures but increases when the temperature is raised or lowered.

During recent years, the conclusions drawn from studies on bacterial luminescence have been found to be applicable to many phenomena, such as growth, disinfection, cardiac rhythmicity, contraction, cell division, and amoeboid motion. It seems certain that knowledge brought to light in this long series of studies may have an important bearing on the role of chemical agents in induced hypothermia. To allay any doubts, the results of Overton on the anesthetization of tadpoles by ethyl alcohol may be mentioned. Here tadpoles, anesthetized at 20° C., tend to revive on being cooled. Since this is the typical action of a Type II compound, Johnson and Flagler (1951) argued that compression by reducing the volume of the protein enzymes should revive the animals and proceeded to perform experiments to test the matter. The results were as expected: on compression, anesthetized salamander larvae resumed swimming, and on subsequent decompression they became inactive.

The results of this simple but critical experiment give clear evidence that reactions, similar to the luminescence reaction in their basic physicochemical relations, are involved in the responses of a vertebrate to temperature and anesthetics.

Another sort of reaction, differing from the luminescence type only in that the

reversible thermal denaturation is absent, is typical of many cellular processes and enzymic reactions. In these the logarithm of the rate increases linearly with the reciprocal of the absolute temperature until irreversible thermal denaturation ensues more or less abruptly at a temperature near or above the upper physiological limit. Since the reversible thermal denaturation is absent, inhibitors (Type I) act primarily by combining with the prosthetic group, competing with the substrate at this site. As a result, their potency tends to increase progressively with a lowering of the temperature.

Serum cholinesterase exemplifies the above type of process. In the absence of an inhibitor the rate of the cholinesterase of dog or human blood serum varies with temperature in accordance with the Arrhenius relation, beginning to show some irreversible denaturation above 30° C. (Bach, *et al.*, 1951; Robert, *et al.*, 1951). When an inhibitor such as quinine is present, the inhibition at low temperatures is much greater and the temperature coefficient is increased accordingly. It is significant, however, that if a series of quinine derivatives is compared the degree of temperature sensitivity varies widely, the inhibition of certain members being independent of temperature over the physiological range (Lawler, Brown, unpublished). An anticholinesterase with the latter characteristics would be particularly useful in the regulation of induced hypothermia.

The foregoing physicochemical considerations are sufficient to illustrate the types of reactions underlying cellular processes and to show the manner in which they are influenced by temperature and chemical agents. In induced hypothermia, under conditions of a natural circulation, the chemicals subject to experimental control are drugs and such substances as might be used in an attempt to sustain the electrolyte balance. In relation to their use, the above discussion may serve to emphasize the fact that one group of substances (Type I) combines with the prosthetic group of the enzyme and is usually potentiated by a lowering in temperature, but it is possible to have inhibitors that act independently of temperature. The additional fact is that enzymes which exhibit a reversible thermal denaturation are also inhibited by substances such as ether and narcotics (Type II), the inhibition tending to decrease with a lowering of the temperature. This could be a disturbing factor in hypothermia but could be eliminated if anesthetics acting independently of temperature could be employed.

In turning to a consideration of cellular processes, such as excitability, rhythmicity, contractility and secretion which may be the target of metabolites and other agents in hypothermia, it may be stated that they reflect the behavior of enzymatic systems in their temperature dependence. This is shown in the case of the cardiac rhythm which Landau and Marsland (1952) have found to be controlled by a process showing a temperature and pressure dependence resembling in its main characteristics the luminescence reaction. The contractility of auricular strips of the turtle also shows a dependence on events which increase with temperature to an optimum and then decrease, the latter decrease, as in the case of luminescence, being reversed by high pressure.

The major problem with which we are faced in dealing with such complex cellular processes is insufficient knowledge on which to decide whether the potentiation of some processes results from the inhibition of a recovery process or an increased

activation. Or for that matter whether a reduction in activity with increasing temperature depends on the increase in rate of some interfering reaction or a reversible thermal inactivation of a controlling enzyme. In the former a Type I inhibitor could lead to an increase in activity while in the latter a Type II would increase the inhibition. Clearly the extent to which the physical chemistry of enzymes may be applied to cellular processes is limited by our knowledge of cellular physiology.

In hypothermia considered at the cellular level the impairment of any cellular activity arises when the energy essential for function is impaired either for the primary cause of anoxia or the secondary cause of an insufficient electrolyte balance across the cell membrane, both stemming from an inadequate composition of the extracellular fluid. In the light of the body of evidence on cellular function now available, it seems certain that the locus of action of the above agents is on the cell membrane. By acting there they tend to limit its capacity (a) to maintain the electrical potential, (b) to excite, (c) to induce activation, and (d) to determine the duration of the active state. Since the cardiac contractility is of such importance in hypothermia it is appropriate to consider its temperature dependence and the extent to which it depends on excitation and the activation cycle.

The significant fact concerning the effect of temperature on the isometric tension developed by an isolated strip of heart muscle is that all vertebrates exhibit a temperature optimum. Thus the frog, turtle and cat have optima at 0° , 10° and 22° C., respectively, the tension diminishing at lower or higher temperatures. In an unfatigued heart the tension developed at the optimum temperature is the maximum which can be developed at any temperature and pressure or in the presence of drugs such as β strophanthin. If the rate of stimulation is increased above a certain limit, the tension at temperatures below or at the optimum is reduced. But above the optimum temperature, where treppe exists, the maximum tension may be attained provided a suitable rate of stimulation is employed at each temperature (Hajdu and Szent Györgyi, 1952; Twente, 1955).

Perhaps the most important fact with respect to tension and temperature is that when the heart is treated with β strophanthin or if the Ca^{++} is sufficiently increased, the tension increases with temperature to the maximum level and maintains this value at increasingly higher temperatures over the physiological range and treppe is non-existent. In the turtle the maximum tension obtains from 9° to 34° C. When the heart is in this state it requires a much higher rate of stimulation before the tension is reduced. In the mammal the maximum tension is reached at about 22° C. and it would be expected that increased Ca^{++} , digitalis, or β strophanthin would cause this tension to be sustained up to 38° C., provided that the rate of beat were sub-optimal.

The view has been held by some that a heart *in situ* under normal physiological conditions does not exhibit treppe. Although this may be so, it is certain that the "treppe state" is readily induced by rather minor changes in the composition of the extracellular fluid and it is quite probable that it would appear during progressive hypothermic failure. If this were the case, it would be a very unfavorable situation since the isometric tension becomes very dependent on heart rate. The fact that digitalis, alkaloids, cortisone and other agents tend to stabilize the tension with

respect to rate and temperature warrants the further investigation of their potentiality in hypothermia.

A very important reason for considering the treppe state is that recent studies have shown that it depends upon events occurring coincident with the brief period of activation of the heart.

The treppe studies of Twente were based on observations (Brown, unpublished) on the manner in which high pressure increases the isometric tension of the auricular muscle of the turtle. Here it was shown that the greater tension developed under pressure was produced *only* if the muscle was under compression during the latent period and the initial one-tenth of the contraction phase. Pressure applied at the end of this period and sustained throughout the contraction did not increase the tension. Since the pressure-effective period coincides approximately with the QRS complex of the action potential, it seems clear that some process restricted to this period is augmented by pressure.

Recently Twente demonstrated that at 20° C. pressure increased twitch tension to the greatest extent (100%) in a non-beating auricular strip capable of treppe. If the maximum treppe tension is allowed to develop by stimulation at the optimum rate, pressure has only a slight effect (10%). When the treppe condition is eliminated by treatment with β strophanthin the tension is not altered by pressures up to 10,000 psi.

The conclusion to be drawn from the foregoing is that pressure increases the tension by eliminating the treppe condition. Since the pressure-effective period coincides with the QRS complex of the action potential, it follows that treppe also depends on events restricted to this period. Further, since β strophanthin eliminates both treppe and the action of pressure, it may be concluded that the action of this agent is also on events restricted to this period in the cycle.

On the basis of the pressure data on tension in striated muscle it was proposed earlier (1941) that stimulation induces an alpha process which reaches a maximum within the first one-tenth of the contraction cycle and then diminishes. The change in state set up by the alpha process then causes activation of the actomyosin and the development of the active state. It was considered that under pressure the alpha process was increased and therefore a larger tension developed.

On the basis of the heart studies it may be concluded that the above explanation is applicable to this tissue and that the alpha process reaches a maximum within the QRS interval of the action potential. In terms of the preceding, it would be concluded that pressure and β strophanthin acted on the alpha process, increasing its magnitude, while the treppe condition would depend on a decrease in this process.

According to the proposal of Hill (1949), the tension developed in the isometric twitch is determined by the duration of the "active state." Clearly pressure does not directly affect the rate of de-activation since compression applied at the end of the alpha process and maintained throughout the remainder of the contraction cycle fails to increase the tension. From this it may be concluded that the magnitude of the alpha process determines the duration of the active state and that pressure, treppe and β strophanthin, by modifying this process, influence the development of tension.

The coincidence of the alpha process and depolarization of the muscle suggest that an increase in the alpha process may represent a longer persistence of the state of

depolarization. This would permit a greater sodium shift and lead to the creation of a greater activation potential. Such changes in the intracellular ion concentration would be secondary to the membrane changes accompanying depolarization. The basic problem then is the nature of the reactive process which determines the period of membrane depolarization.

The purpose of presenting the foregoing somewhat detailed consideration of heart muscle was to give an example of the type of system which requires intensive physicochemical study and to indicate one way in which it may be investigated. It has been shown that the reactive state of the cell surface can influence the duration of the active state and the size of the contraction, that it is subject to the action of temperature and pressure, and that substances such as β strophanthin in very small concentration can give great stability to the system. A concerted attack on the physical chemistry of this interfacial activator system, particularly in the direction of identifying more effective stabilizing agents, would be a worthwhile effort. From such a program there might come methods of regulating more effectively the activities of cells in hypothermia and other conditions.

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EFFECTS OF HYPOTHERMIA ON GENERAL METABOLISM*

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Survival of isolated cells, tissues and small organisms after cooling and storage at very low temperatures, close to absolute zero, has been frequently reported. In contrast, adult intact animals apparently are sensitive to moderate reductions in body temperature. In dogs and man body temperatures below 25° C. usually contraindicate surgical procedures because of the risk of ventricular fibrillation and cardiac arrest. However, temperatures as low as 18–19° C. have been induced in dogs and man with survival.^{1, 2, 3} Rodents have been frequently cooled and successfully revived from body temperatures of approximately zero.^{4, 5, 6} Recently Gollan⁷ using extra-corporeal cooling, oxygenation and rewarming of the blood has revived dogs with body temperatures as low as 0° C. and in cardiac arrest for one hour.

Marked species variation in the response to hypothermia has been demonstrated.⁸ This has been expressed for some species as mean duration of survival time consequent to exposure to a constant air temperature of –35° C. Under these conditions the mouse survives 0.4 hours, the rat 0.75 to 2.0 hours, the rabbit 3.5 to 6.5 hours, and the pigeon 22 to 78 hours. Smaller members of a species respond better to a lowering of their body temperature than do the larger ones. Similarly, a significant difference was noted in the lethal temperature of adults and infants of various species.⁹ Maquire and Merendino¹⁰ have also remarked upon the greater tolerance of the younger animals. Furthermore, they reported that cardiac arrest occurred in their juvenile dogs while ventricular fibrillation occurred in the adults. All of these observations are suggestive of the complexity of an organism's response to lowered body temperature.

Every biological process depends upon a series of consecutive reactions, each characterized by a definite temperature coefficient and each becoming the limiting factor or the master process at a definite temperature. This implies that certain reactions have a more significant influence at some temperature ranges than others. This does not deny the importance of other factors such as the products of reactions, the chemical environment or the availability of the substrate on chemical or biological processes or responses. In fact, these other factors are to some extent determined by temperature. All of these influences can be summed in the Arrhenius formula, wherein

$$K_1 = K_0 \frac{u}{eR} \left(\frac{T_1 - T_0}{T_0 T_1} \right).$$

This equation reflects the underlying metabolic chemical reactions. Activation energies calculated from this formula are of the order of 11,500 and 16,500 calories and are those associated with cell respiration corresponding to hydroxyl and iron catalyzed systems respectively. Therefore, it is evident that oxygen is a limiting factor and temperature a controlling factor in the response of the cell and the organism to their environments.

Efficiency of cellular function is determined by their metabolic composition con-

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sequent to prior activity. These metabolic gradients are predicated upon enzyme mosaics. Enzyme activity is modified by temperature but the enzymes found in a cell may not have the same thermo-activity characteristics. Therefore, the relative inactivity or stimulation of members of the system alter the substrate and the concentration of metabolic end products and consequently derange the system. This suggests that the exact temperature of cells and the influence of this temperature level on the components of the cell are the most important factors to be considered in the evaluation of the animal's response to a lowered (basically an altered) body temperature.

There are considerable differences in the temperature of different parts of the body core and body surface (fig. 1).¹¹ Whether similar gradients exist in the hypo-

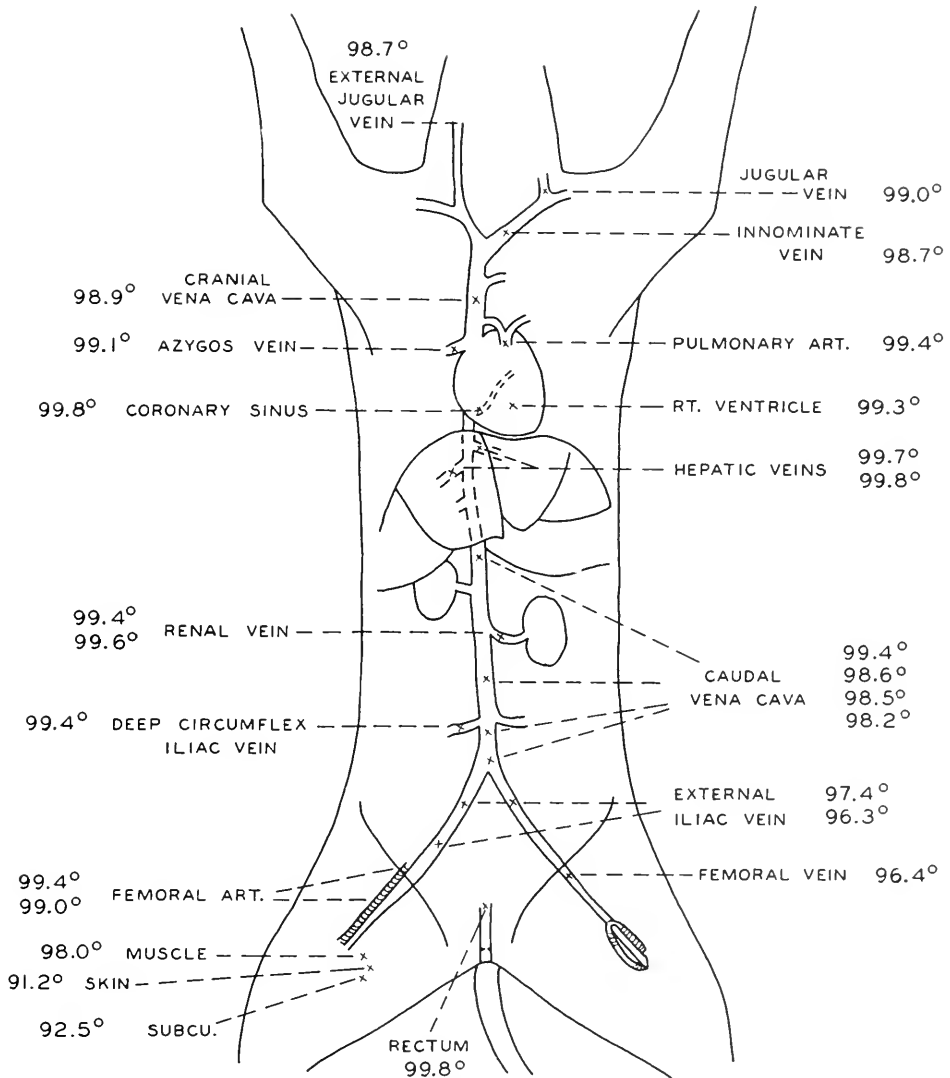


FIG. 1.—Thermal gradients in blood and various tissues of the dog.
Ambient temperature = 22.2° C.

thermic animal has not been determined. Unpublished observations by the authors¹² indicate that they do exist but that the direction of the gradient in certain areas is reversed. Observations on right heart and rectal temperatures show a slight discrepancy of 1° C. This difference becomes greater at rectal temperatures below 20° C. because the heart cools more slowly than the rectum. Hart¹³ has measured the heat content of small animals by a calorimetric method and in hypothermia has found rectal temperatures to be lower than average body temperature. In non-chilled animals average body temperature is 1 to 2° C. below rectal. These few indications of variable temperatures in the hypothermic and non-chilled animal are indicative of the unstable background upon which any discussion of hypothermia rests. In view of the influence of temperature upon enzyme activity, the dissociation of water, the pH at neutrality, the isoelectric points of proteins, the balance of electrolytes between cells and surrounding fluid, and many other biological phenomena, the simple assumption of rectal temperature as indicative of a thermal state is likely to lead to unjustified conclusions. Alterations in the chemical balances of the cell that may be innocuous at the cell's optimum temperature whether it be 20° or 40° C. may prove to be disastrous if the temperature be only slightly altered. Furthermore, if two adjacent units adapted to operating at a 5° or 10° C. differential are as a consequence of hypothermia asked to perform at identical and lower temperatures their responses and those of other units may not be in adequate harmony for the best interests of the total organism.

However, most of the available data relating energy output and temperature have been referred to temperatures obtained from the rectum. Consequently, discussion of the finer details of temperature and metabolic activity must be ignored. In a hibernating animal with a body temperature of approximately 4° C., the oxygen consumption drops to 3 to 10 per cent of normal. Considerable variation exists as to the degree of depression, since it is quite difficult to secure many adequate observations, due to technical difficulties. Early studies upon the oxygen consumption of humans (body temperature 28° C.) and dogs, rats and rabbits (as low as 19° C.) have given variable results. Values ranging from 300 per cent increase to 50 per cent decrease have been recorded.^{14, 15, 16, 17, 18, 22} The high values have been attributed to the increased muscular tension and shivering invoked by cold. Bigelow *et al.*¹⁹ reported that oxygen consumption fell consistently with reduction in body temperature (to 18° C.) and rose in proportion during rewarming. According to Lynn *et al.*²⁰ the reduction in oxygen consumption is 27 per cent at a rectal temperature of 30° C. and also it decreases linearly with temperature. Extrapolation to zero oxygen consumption would place the rectal temperature at 10° C. Similar relationships for the rat were noted by Adolph¹⁸ and earlier by Woodruff²³ for the dog.

An exponential decrease in oxygen consumption and carbon dioxide production with decrease in the rectal temperature was reported by Velten.²¹ A similar relationship was observed by Spurr *et al.*^{1, 2} and could be expressed by the equation

$$\log y = 0.37x - 0.6926.$$

Their data gave Q_{10} values of 2.3 in good agreement with van't Hoff's law. If the animals were shivering during the development of the hypothermic state, the oxygen consumption followed the same pattern of response to reduction in rectal temperature but at a significantly higher level (fig. 2).

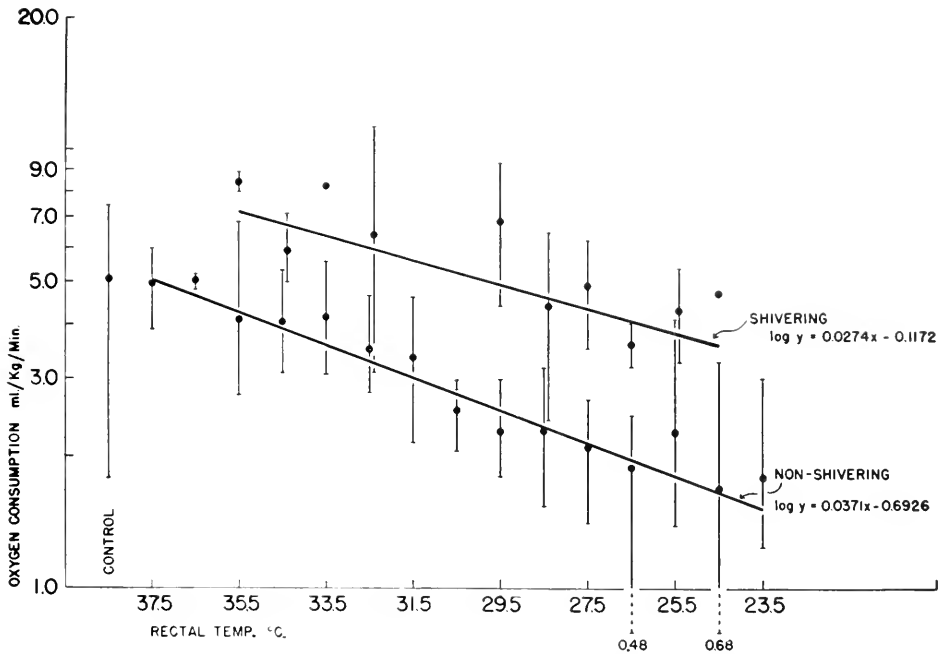


FIG. 2.—Oxygen consumption, under condition of shivering and in the absence of shivering, during progressive reduction of the rectal temperature in 24 experiments on 19 dogs. Points indicate the mean values and vertical lines the extremes of variation at each temperature level.

The level of activity of oxygen consumption as well as other physiological functions of the hypothermic animals shows an exponential decline with decreasing body temperatures. This decline is not greater than expected and can be expressed as being physiologically adequate for the situation existing at the time.

Considerable attention has been given to the problem of spontaneous versus controlled respiration. Oxygen consumption in relation to body temperature does not appear to be affected by the mode of ventilation of the lungs.²⁰ Oxygen supplies to the tissues apparently are adequate in either instance. The major point of controversy hinges not upon any metabolic differences but upon the relative incidence of ventricular fibrillation observed. Spurr *et al.*²⁴ did not find a greater incidence of fibrillation in their spontaneously breathing dogs than other investigators who employed controlled respiration.

Oxygenation of arterial blood even when breathing room air appears to be fully adequate.²² However, Dill and Forbes¹⁶ have noted a decrease in arterial pO_2 from 98 mm. Hg. at 37.1° C. to 70 mm. at 27.8° C.† This decrease was attributed to altered rates of diffusion through lung membranes and was supported by the finding of pulmonary edema in one patient and on Walther's²⁵ demonstration of pulmonary edema in cooled rabbits. This anoxemia, evidenced by cyanosis and relieved by oxygen inhalation, was also noted by Woodruff²³ who similarly found pulmonary edema in one of his dogs. Although development of a state of anoxia during hypo-

† At 20° C. approximately 32 per cent more oxygen is physically dissolved in plasma than at 38° C.

thermia has been assumed to occur by some investigators, Noell²⁶ reported that the EEG changes observed were not typical of anoxia but were similar to eserine poisoning. He suggested that the breakdown of acetylcholine might be delayed at low temperatures. It should be noted that Dill and Forbes¹⁶ found no evidence of arterial unsaturation since the oxygen dissociation curve shifted sufficiently to the left to maintain a normal arterial saturation.

This shift in the oxygen dissociation curve to the left with lowered blood temperatures is not as disastrous an event as many investigators have believed. Concomitant with this tendency, accumulation of CO₂ has been demonstrated to result in a respiratory acidosis. This state of acidosis results in a shift of the oxygen dissociation curve to the right. In consequence of these opposing shifts of the oxygen dissociation curve, the resultant curve may be relatively normal in shape. Over-ventilation, as practiced by some investigators, lowers the blood CO₂ levels, leaving the temperature effect on the oxygen dissociation curve in command, so that some interference with oxygen exchange may result.

The ventilation equivalent was reported by Dill and Forbes¹⁶ to be increased. The respiratory volume of most of their patients was large in comparison with the volume of oxygen removed. Normal persons at rest remove from 3 to 5 per cent of oxygen from inspired air and this proportion is not altered greatly during moderate activity. This observation has not been confirmed in the hypothermic dog² but the discrepancy may lie in the marked shivering and high oxygen consumption in the patients of Dill and Forbes (fig. 3). It should be emphasized that the respiratory center responds normally to CO₂ at low body temperatures.²⁷

Finney *et al.*²⁸ found a marked fall in the R.Q. with reduction in the body temperature. The R.Q. was observed to fall as low as 0.32 provided that shivering had been suppressed. Even during shivering the R.Q. could fall below 0.70. Similar results have been found by the present authors and Gray *et al.* (communicated today) observed a low R.Q. in isolated cooled livers.

The reduction in oxygen consumption of the total organism with lowered body temperatures is apparently shared by all organs. This has been observed for the brain, kidney and liver.^{29, 30} Whether any of these or other tissues have a disproportionately greater or smaller change in oxygen utilization cannot be evaluated on the basis of presently available data.

The patterns of heat exchange have not been completely analyzed during the development or maintenance of the hypothermic state. Heat losses are enormous at the beginning of cooling despite the increase in metabolism.^{1, 2, 18} Analyses of the pattern of heat losses during a steady state of lowered body temperature are in progress but definitive conclusions are not as yet available (figs. 4, 5, and 6).

Lyman and Chatfield³¹ have reviewed the changes which occur in protein and fat metabolism during hibernation but these variables have not been followed in the hypothermic non-hibernator. Finney, Dworkin and Cassidy²⁸ found that hypothermia resulted in a diminished respiratory quotient in non-shivering dogs and an increased quotient when the animals were shivering. They thought that this represented a change in the character of the animal's metabolism. However, in the light of present knowledge of carbon dioxide retention and elimination in hypothermia and shivering, this conclusion is not completely justified.

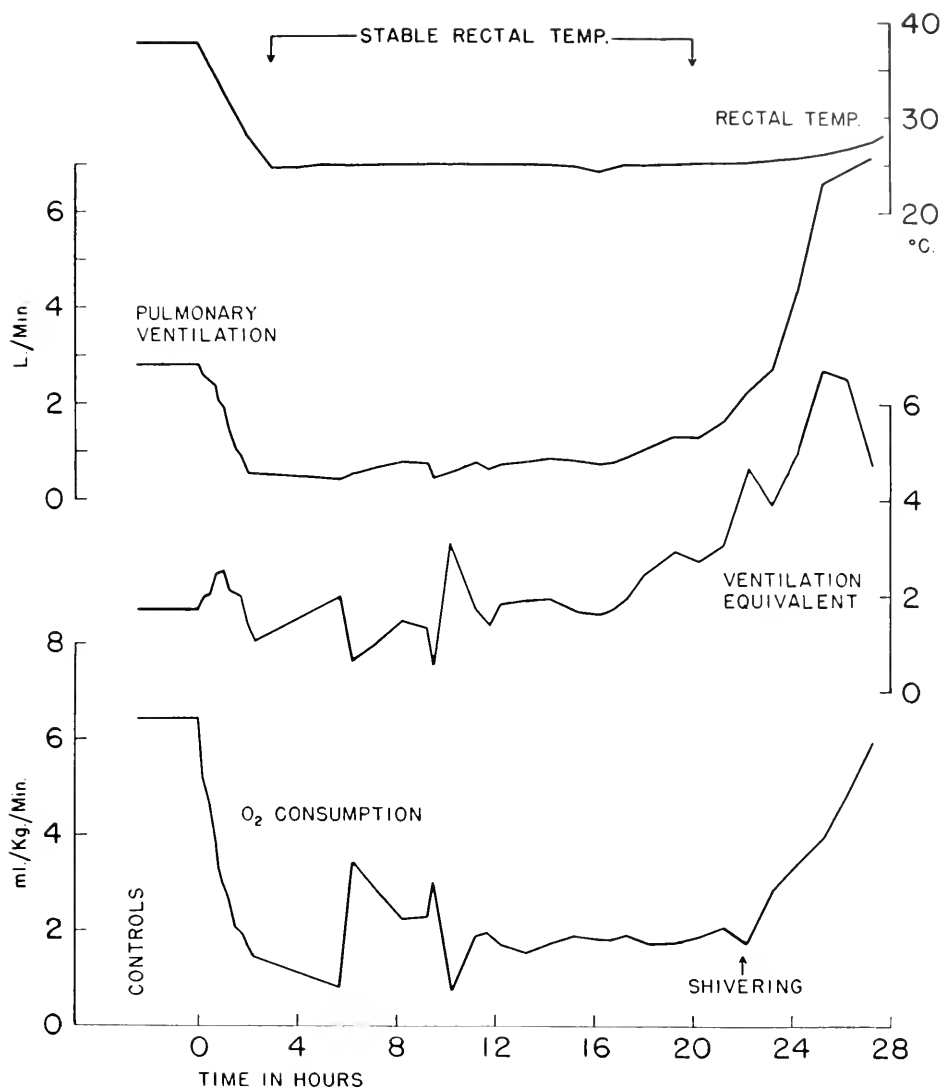


FIG. 3.—Rectal temperature, pulmonary ventilation, ventilation equivalent and oxygen consumption of an animal that was maintained at a rectal temperature of $25.0 \pm 0.1^\circ \text{C}$. for 17 hours before rewarming occurred.

The effect of reduced body temperature on carbohydrate metabolism has been studied to some extent. According to Fuhrman and Crismon³² hyperglycemia in animals cooled to about 20°C . was noted as early as 1855 by Claude Bernard. Other investigators have also reported increased blood sugar as a result of hypothermia.^{33, 34} It has been demonstrated that lowering of the blood sugar by insulin injection resulted in the cessation of shivering in cooled dogs and that administration of glucose caused its reappearance.³⁵ Fuhrman and Crismon³² found that in shivering rats provided with ample carbohydrate previous to cooling, blood glucose levels increased during the initial phase of hypothermia, associated with a concomitant decrease in

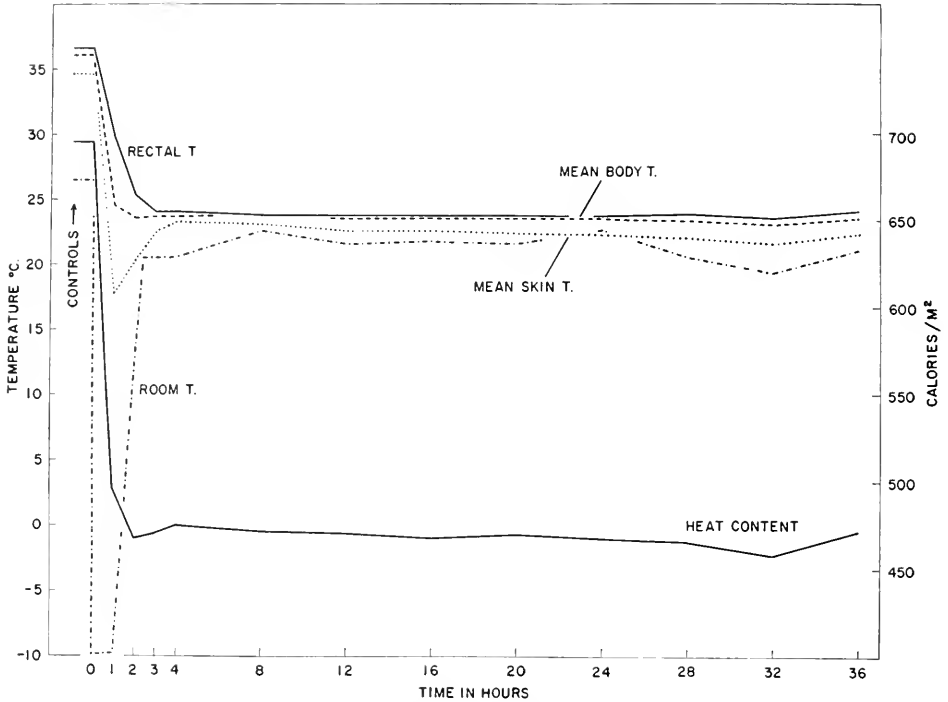


FIG. 4.—Rectal, mean skin, mean body temperatures and heat content of a dog that was stable at a rectal temperature between 23.5–24.0° C. for 34.1 hours. At the end of this time ventricular fibrillation occurred and death ensued.

liver glycogen. The blood glucose then remained elevated throughout the remainder of the hypothermia. Furthermore, in starved animals or slowly-cooled fed animals the blood glucose was maintained or fell during cooling. Slow cooling for prolonged periods has been demonstrated by others to result in hypoglycemia.^{33, 36} The evidence therefore seems to indicate the necessity of differentiating between shivering and non-shivering animals during the initial stages of cooling, and between chronic and acute hypothermia. Also, the carbohydrate stores of the animal must be considered. In the presence of shivering, provided the animal's source of carbohydrate is adequate, blood glucose increases. In acute hypothermia further reduction of body temperature results in cessation of shivering and a consequent reduction in metabolic rate and utilization of carbohydrate. Thus, the early hyperglycemia is maintained.³² If the reduction in body temperature is prolonged, shivering persists with a consequent exhaustion of liver and muscle glycogen and a resulting hypoglycemia. In the absence of shivering during cooling, the blood glucose level may be maintained or decreased.³²

Necrosis of depot fat may be caused by exposure to cold. Hocksinger³⁷ and Haxthausen³⁸ have reported cases of subcutaneous fat necrosis in children following exposure to low temperatures. This tendency to develop fat necrosis may be dependent upon the chemical composition of the fat present. The melting point of subcutaneous fat of children is much higher and has a lower iodine number than that

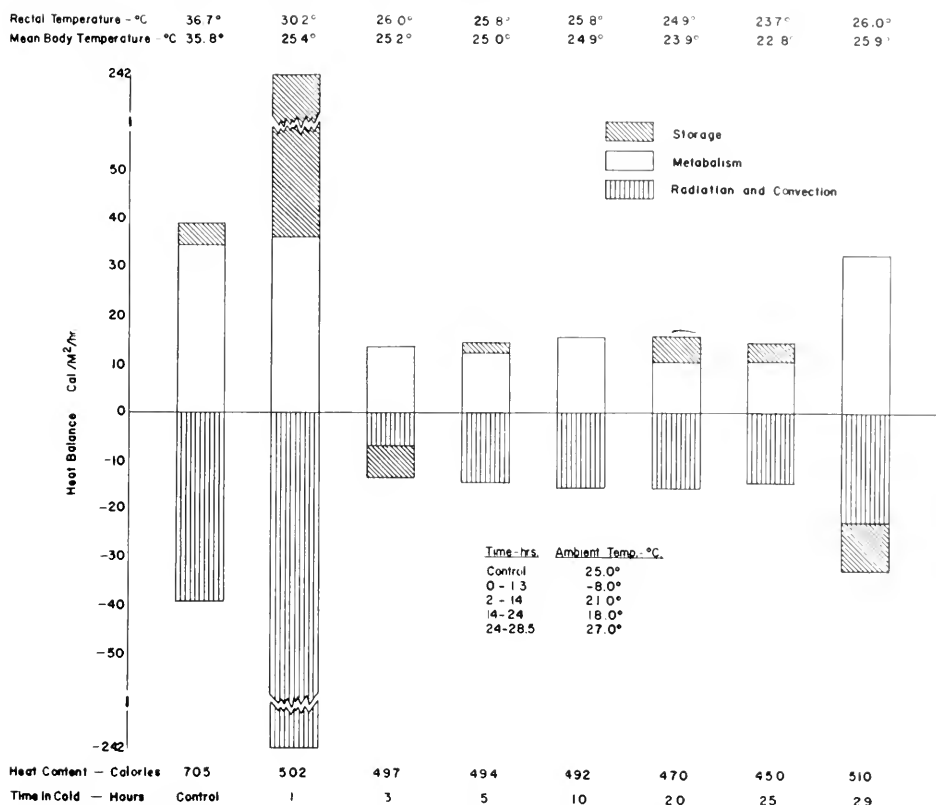


FIG. 5.—Heat balance of a dog that was maintained at a rectal temperature between 23.5–26° C. for 24 hours with subsequent recovery. This animal was breathing 100 per cent oxygen spontaneously throughout the course of the experiment. Heat content should read Cal./M².

of adults. The hypothermic cooling of these fat layers and masses to below the solidification point may lead to this necrosis. Several investigators have noted during the progress of hypothermia the presence of isolated hard tissue masses and have felt that these represent "frozen areas." They may well have been nothing more than solidified fat masses. It is interesting that the fat of animals who successfully hibernate has unusually low solidification temperatures, namely, their fats remain fluid at low temperatures. This implies the presence of body constituents of unusual physicochemical properties. The use of diets leading to a greater quantity of unsaturated body fats may be a valuable adjunct to hypothermic therapy.

Since the development of hypothermia is usually accompanied by the administration of various drugs, it is necessary to evaluate the relationship between drug action and temperature. Whenever a drug has a high temperature coefficient of action, slight variations in body temperature may be profoundly significant. Alcohol demonstrates this fundamental concept. At temperatures near the normal optimum, a slight rise in temperature markedly increases the potency of a given concentration and conversely a slight fall in temperature decreases the potency. Similarly, the quantity of insulin required to induce convulsions in mice whose body temperature is dropped

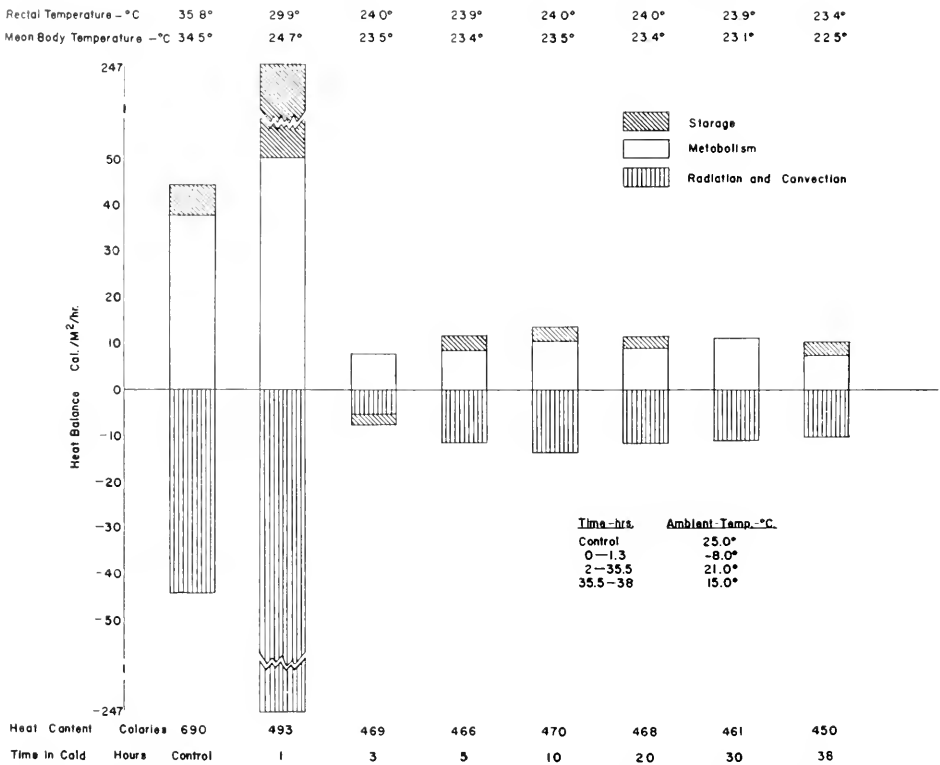


FIG. 6.—Heat balance of the dog in fig. 4. This animal was breathing room air spontaneously throughout the course of the experiment. Heat content should read Cal./M².

only 4° C. is some 90 times greater than that required at normal body temperatures. Cassidy *et al.*³⁹ demonstrated a delay in the drop of the blood sugar following insulin administration in dogs cooled to 25° C. The enzymatic detoxification of epinephrine is slower at low temperatures, a given dose resulting in a longer and more intense action during hypothermia. This is in contrast to studies on isolated tissues where epinephrine effect is greatest at temperatures of approximately 37° C. Substances which are chemically detoxified by the tissues may have their action prolonged by hypothermia while drugs not detoxified may be uninfluenced by the temperature change. A number of factors must be considered before critical evaluation of drug action can be made. The accumulation of such data must take into consideration such matters as rates of absorption and distribution to effector areas, permeability changes (selective) of the cell, rate of destruction, effective ratios of concentration of drug and substrate at point of action, response of tissue to concentration and the intracellular enzymatic mosaic alterations resulting from changes in local temperature. This local temperature effect may also be modified by the differentiated action of temperature upon adjacent cells which may respond in an entirely different manner.

ELECTROLYTES

Sodium. Swan *et al.*,¹⁰ Fleming,¹¹ McMillan *et al.*,¹² and Deterling *et al.*¹³ have reported that the concentration of serum sodium does not change appreciably during hypothermia. Da Costa *et al.*¹⁴ also observed no change in cyanotic dogs subjected to hypothermia. Osborn¹⁵ found that, in general, serum sodium decreased in concentration though no values were reported.

Potassium. The changes which occur in the plasma or serum concentrations of potassium during hypothermia are less clear-cut than for sodium. Fleming,¹¹ Deterling *et al.*,¹³ and Da Costa *et al.*¹⁴ found no significant change in plasma potassium at low body temperatures. McMillan *et al.*¹² also found little change in plasma potassium either for dogs which were respiring spontaneously during the hypothermia or for dogs in which artificial respiration was maintained at 24 cycles/min. throughout the cooling procedure. The latter group of animals demonstrated an average decrease in potassium to about 85 per cent of the control value (3.5 to 3.0 mEq/L.). This rate of ventilation, at low body temperatures, would represent a hyperventilation of the animals. There was a progressive washout of carbon dioxide to as low as 12 vols. per cent at rectal temperatures of 22° C. However, Swan *et al.*¹⁰ found that hyperventilation alone (50–60 cycles/min.) produced a decrease in potassium to about 70 per cent of control.

Bigelow, Lindsay and Greenwood¹⁶ found a consistent increase in serum potassium concentration in dogs and Elliot and Crismon¹⁷ reported that the cooled rat demonstrated a rise in serum potassium from a mean value of 3.55 ± 0.64 to 5.28 ± 0.70 mEq/L. at rectal temperatures of 25° C. Since potassium did not increase to a toxic level, the question arose regarding altered sensitivity of response in hypothermic animals. The latter authors found that injections of potassium salts which were well tolerated by the normothermic rats resulted in fatal potassium poisoning when injected into hypothermic rats. If the hypothermic animals were given oral glucose or injection of calcium chloride or ouabain previous to the potassium injections, protection against the lethal effects of the latter was obtained. Fenn¹⁸ had found previously that the storage of glycogen in the liver involves an increase in both hepatic water and potassium. When release of the previously stored carbohydrate occurs there is a simultaneous release of water and potassium. Fuhrman and Crismon¹⁹ had reported a sharp lowering of liver glycogen in cooled rats and Samaras²⁰ found that an effective method of rendering the dog's liver glycogen-free was the induction of shivering by exposure to cold. Combining these results, Elliot and Crismon¹⁷ attributed the rise in serum potassium observed in their experiments, at least in part, to the release of potassium by the breakdown of liver glycogen, and the protection afforded by oral glucose to the maintenance of a high liver glycogen. Furthermore, muscular activity is known to deplete muscle of potassium²¹ and to bring about an increase in plasma potassium.²² Consequently, part of the increase in potassium seen in hypothermia may be due to the increased muscular activity of shivering.

Swan *et al.*¹⁰ found a consistent decrease in serum potassium in hyperventilated dogs during hypothermia. They reasoned that there was a shift of potassium to the intracellular phase since the loss could not be accounted for in the urine. During oc-

clusion of the general circulation potassium appeared to accumulate in the tissue spaces. Upon resumption of flow, potassium levels increased rapidly in the serum and the concentration after occlusion was greater than before occlusion. These authors were successful in converting ventricular fibrillation in hypothermic dogs to a normal beat by the injection of potassium chloride followed by calcium chloride.

Osborn⁴⁵ also reported a decrease in serum potassium in hypothermia and Brewer⁵¹ found that the depression of body temperature following decerebration was associated with a depression in the plasma potassium level. He observed further that increases in body temperature brought about by dinitrophenol resulted in increased plasma potassium levels. If the dinitrophenol was given to the decerebrated preparation no decrease in body temperature or plasma potassium occurred. Furthermore, the stimulus of low environmental temperature, producing an increase in general body metabolism, increased the plasma potassium in both normal and decerebrated animals. Brewer concluded that there was a rather good positive correlation between metabolic activity and the plasma potassium level.

It appears that several factors must be taken into account when considering plasma or serum potassium levels in hypothermia. The observation that the release of carbohydrate from the liver is associated with simultaneous release of potassium,⁴⁵ together with the finding that the liver glycogen is rapidly utilized during hypothermia and that blood glucose increases during the early phases of hypothermia and remains elevated in previously fed rats,³² indicates the necessity of delineating the nutritional status of the animal at the time the experiments are performed. In rats which were starved previous to the induction of hypothermia the blood glucose was maintained or fell during the course of cooling.³² The rate at which animals are cooled may also play a role since slowly-cooled, fed rats exhibit the same blood glucose picture as rapidly cooled, starved animals.³² The presence or absence of shivering as a sign of muscular activity and the severity of the shivering response must also be considered, since it has been demonstrated that increased muscular activity is associated with loss of potassium from the muscles and increased blood levels.^{49, 50} The finding of Swan *et al.*⁴⁰ that hyperventilation alone results in decreased potassium, together with their observed decrease in potassium levels in hyperventilated, hypothermic dogs, suggests that the ventilation of the experimental animals must be considered. Mackay's⁵² findings were suggestive of a correlation between hypercapnia and potassium but McMillan *et al.*⁴² were unable to demonstrate any correlation between these variables during hypothermia. At present the relationship between CO₂ content and serum potassium is not clear.

Calcium. Elliot and Crismon¹⁷ found a statistically significant increase in rat serum calcium levels upon reduction of the temperature to 25° C. This was associated with an increased potassium level but the Ca/K ratio was reduced from 1.46 to 1.08. Bigelow *et al.*⁴⁶ reported an increased serum calcium in 3 out of 4 experiments on dogs. McMillan *et al.*⁴² also observed an increased serum concentration of calcium in both spontaneously respiring and artificially respired dogs. They determined free and bound calcium and, with the exception of the bound calcium in artificially respired animals, observed progressive increases down to rectal temperatures of 19° C. Since they also observed little change in potassium, the Ca/K ratio increased, in contrast to the findings of Elliot and Crismon.¹⁷ On the other

hand, Fleming¹¹ found no significant change in plasma concentrations of calcium at rectal temperatures of 20° C.

The role of calcium in hypothermia is undefined at present but may be of importance because of its well known power of offsetting the toxic properties of potassium.⁵³ In this connection, the protective effect of injections of calcium against subsequent administration of potassium to hypothermic rats has already been mentioned.¹⁷ The literature on the effects of calcium on the physiology of the cell is voluminous.⁵³

Magnesium. Platner and Hosko⁵⁴ observed a linear increase in serum magnesium with reduction of the rectal temperatures of the dog, cat, rat, hamster, and turtle. On the basis of Heilbrunn's statement that cold "releases calcium into the cell interior" they postulated that magnesium ions were thereby displaced and passed not only to the cortical protoplasm, but out of the cell. Contrary to these findings are those of Fleming¹¹ and McMillan *et al.*¹² who observed no significant change in the serum magnesium of the dog during hypothermia. However, these observations were made at much lower rectal temperatures than those obtained for the dog in Platner and Hosko's experiments. It may be that there is an initial increase in serum magnesium in the dog followed by a reduction to near control levels as the rectal temperature is progressively reduced. However, this does not explain the increased serum magnesium levels observed in other homiothermic animals.⁵⁴

Cations. Chloride, phosphate and bicarbonate have been determined during hypothermia by several investigators. Swan *et al.*⁴⁰ found no change in serum chloride that was not ascribable to the hyperventilation, Fleming,⁴¹ Deterling *et al.*⁴³ and Woodruff²³ observed no significant change at low rectal temperatures while Osborn⁴⁵ reported a slight decrease. Earlier, Barbour, McKay and Griffith⁵⁵ described an initial increase in serum chloride upon cooling of monkeys and rats to rectal temperatures of about 30° C. This initial peak was preceded by a depression which the authors believe may have been the result of an epinephrine effect. Upon further cooling of the animals to 23° C. the serum chloride concentration approached or attained the control levels. This is in agreement with the finding of no change from control levels after variable periods of time below 27° C.²³ and no change consistently at rectal temperatures of 20° C.⁴¹

The serum phosphorus was found to decrease slightly by Swan *et al.*⁴⁰ who suggested that there may be a movement of both phosphorus and potassium into body cells in association with a disturbance in carbohydrate metabolism. Fleming,¹¹ on the other hand, found no significant change in the serum phosphate at rectal temperatures of 20° C.

It was observed by Osborn⁴⁵ that a low serum bicarbonate was associated with the subsequent occurrence of ventricular fibrillation or with the appearance of a 'current of injury' in the electrocardiogram. By maintaining a high serum bicarbonate throughout the hypothermia he was able to effect complete recovery in some animals from rectal temperatures below 19° C. The effects associated with changes in the serum bicarbonate appear to be directly related to pH changes rather than to bicarbonate *per se*. This will be discussed later. Fleming¹¹ reported an increase in bicarbonate at rectal temperatures of 20° C. which were not great enough to compensate for the concomitant retention of CO₂. He injected sodium bicarbonate to

reduce the acidosis but was unable to reduce the mortality rate and frequency of cardiac irregularities.

ACID-BASE BALANCE IN HYPOTHERMIA

The pH of the blood has received considerable attention in investigations concerned with the physiology of hypothermia. It is well known that blood cooled *in vitro* becomes more alkaline.^{56, 57} However, the response is not as simple for *in vivo* blood which is under the influence of respiratory and urinary functions. The results obtained by various investigators are complicated by the type of artificial respiration, or lack of it, employed in their experiments. Thus hyperventilation, hypoventilation and spontaneous respiration each result in relatively different pH changes during progressive hypothermia. In addition, states of artificial respiration which, under conditions of normal body temperatures, may represent hyperventilation, hypoventilation or normal ventilation, may no longer represent these states when the body temperature is progressively reduced. For example, a normal ventilation rate for an anesthetized dog at a rectal temperature of 38° C. is approximately 20 per minute. If an animal is artificially respired at this rate throughout hypothermia the ventilation is no longer normal when the rectal temperature has been reduced to about 25° C. where the 'normal' respiratory rate is approximately 2-3 per minute and the rate of CO₂ production profoundly reduced.² At the lower rectal temperature the animal is being hyperventilated. Furthermore, in the example cited, during the reduction of rectal temperature the ventilation of the animal is progressively changing so that there is a progressively increasing hyperventilation. In the case of the hyperventilated control, at low body temperature the hyperventilation would be relatively greater and hypoventilation at normal body temperatures could represent 'normal' or hyperventilation at low rectal temperatures. It would appear from these considerations that the physiological adjustments which the animal makes, in the acid-base balance of its blood, to reduced body temperatures are best studied in the spontaneously respiring animal. This does not deny the value of studies in which controlled ventilation has been employed. Much valuable information has been obtained from these data concerning the adjustments made by the animal itself, but more particularly concerning the adjustments which can be made for the animal to protect it against various adverse states.

That a definite decrease in blood pH occurs during hypothermia under conditions of spontaneous respiration has been well established by several investigators.^{16, 27, 41, 42, 45, 58} It has been pointed out that in animals in which shivering is allowed to occur during the first stages of hypothermia, there is an initial hyperventilation and consequent respiratory alkalosis. This is followed by a decreased pH to acidotic levels as the body temperature falls and shivering and pulmonary ventilation are progressively reduced.⁴⁵ By hyperventilating dogs and thereby increasing the blood pH, previous to and during hypothermia, Swan *et al.*⁴⁰ found it possible to reduce considerably the incidence of ventricular fibrillation after occlusion of the circulation for 15 minutes. Osborn⁴⁵ also observed that the maintenance of a relatively high arterial pH reduced the incidence of hypothermic ventricular fibrillation. As mentioned previously, he also found a close association between low serum bicarbonate and the onset of severe electrocardiographic changes or ventricular fibrillation. He

reasoned that the initial respiratory alkalosis observed in some of his animals would lower the serum bicarbonate content and render the animals more susceptible to the subsequent respiratory acidosis. Inducing a respiratory acidosis (thereby raising the serum bicarbonate) previous to the induction of hypothermia appeared to have a protective effect in a small series of animals.

McMillan *et al.*⁴² agreed that under conditions of circulatory arrest sudden changes in pH might be the cause of ventricular fibrillation,⁴⁰ but pointed out that this could not be involved in spontaneous hypothermic ventricular fibrillation since pH and CO₂ content change gradually and not suddenly. Covino and Hegnauer⁵⁹ found a marked lowering of the ventricular diastolic and minor dip⁶⁰ thresholds, with consequent ventricular fibrillation, in hypothermia. They ascribed this to the respiratory acidosis which develops at low body temperatures, since both are threshold effects and ventricular fibrillation could be prevented by maintaining the pH near normal during cooling.

The respiratory acidosis which develops during hypothermia would therefore appear to be disadvantageous to adequate cardiac functioning. However, changes in pH and blood CO₂ observed in hypothermia have at least two advantages. Decreased pH tends to counteract the effect of low temperature on the oxygen dissociation curve. Also, the pH and CO₂ represent an attempt to compensate for the depressing action of low temperature on the respiratory center. Cranston *et al.*²⁷ found that in dogs at rectal temperatures of 25–27° C. there was an essentially normal respiratory response to inhalation of 6 per cent carbon dioxide in air and concluded that the observed plasma increase in CO₂ was the result of the action of a functioning control mechanism.

BODY WATER

Relatively little attention has been directed to the question of body fluid shifts which may take place during hypothermia. There have been several isolated observations such as Walther's²⁵ report of pulmonary edema in rabbits which had been subjected to hypothermia and Woodruff's²³ mention of cardiac edema in dogs maintained for long periods at low body temperatures. However, the work of Barbour, McKay and Griffith⁵⁵ on monkeys and rats was the first precise approach to the problem. On the basis of observed changes in plasma proteins and chloride they concluded that there was a decrease in plasma volume and concomitant increase in interstitial and intracellular volumes during the initial phases of cooling. They attributed this movement of water into the cells to the increased metabolic rate of the cells which produced a temporary accumulation of metabolites. Upon cooling to 23° C. the entire water shift was reversed. Rodbard *et al.*⁶¹ pointed out that the data obtained on hematocrit and plasma protein concentration in hypothermia could be obtained without associated changes in blood volume. These investigators found only small changes in hematocrit and plasma proteins in chicks and rabbits subjected to hypothermia. However, plasma volume (Evan's Blue technique) and thiocyanate space were reduced about 30 per cent in hypothermia. Measurement of extracellular volume by means of inulin revealed no changes in this compartment. Inspection of their technique revealed that the measurements of thiocyanate space had been made either before or after the cooling procedure, whereas the determina-

tions of inulin space were made with a single injection before the cooling had begun and again after the animal had been cooled. They interpreted their results as indicating that, while the circulating blood volume was reduced by cooling, the reduction was not due to shift of fluid outside of the circulation. The decreased plasma volume appeared to be due to a trapping of blood in various areas of the vascular bed as a result of the cooling procedure. However, these results do not account for the frequent reports of increases in the hematocrit,^{40, 62, 63} which could only be due to a loss of fluid from the circulating blood or to an addition of cells from the spleen. Swan *et al.*⁴⁰ found an increased hematocrit but little change in the plasma volume, which they believed might have been the result of splenic discharge of red cells.

D'Amato and Hegnauer⁶⁴ noted a transitory rise in plasma protein concentration in dogs at rectal temperatures of 25° C. which was reversed when the cooling continued to 20° C. The consistent decrease in plasma volume observed at the latter temperature suggested that whole plasma was removed from the circulation either by sequestration in constricted vessels or loss to extravascular spaces. D'Amato⁶⁵ later reported a temporary shift of water from the plasma to the tissues of the hypothermic dog. Simultaneously water was found to move from the interstitial compartment of skeletal muscle into the cells. This was associated with shivering. At lower body temperatures, where shivering had ceased, the water movement was reversed. This agrees well with the previous observations of Barbour *et al.*⁵⁵ and with what is known concerning increased intracellular water in skeletal muscle in response to increased activity.^{66, 67}

It thus appears that during the initial stages of hypothermia, and associated with the muscular activity of shivering, there is a shift of water out of the vascular system and a concomitant increase in interstitial and intracellular water. As hypothermia progresses and shivering ceases these water shifts are reversed. The available evidence indicates further that at lower body temperatures the reduction in blood volume is the result of a loss of plasma from the circulation, either by peripheral sequestration in constricted blood vessels or by loss of whole plasma to the extravascular space, or as a response to dehydration and body weight loss.

SUMMARY

A review of metabolic processes during the development and maintenance of the state of hypothermia has been made. Although some general statements could be made it is obvious that the available data are too conflicting to permit much assurance as to the validity of such conclusions. A profound reduction in metabolic processes is agreed to occur. In order for more specific details of the relationship of temperature to metabolic processes to be obtained, a more precise experimental approach must be instituted. Such an approach should incorporate, as a minimum, standardization of at least the following factors: (a) ventilation; (b) anesthesia; (c) nutritional status of the experimental animals; (d) presence or absence of shivering; and (e) rate of cooling, the method employed in cooling, and the final attainment of a steady state.

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THE EFFECT OF LOW TEMPERATURES UPON INTRACELLULAR POTASSIUM IN ISOLATED TISSUES *

I. M. TAYLOR

(Read by E. CALKINS)

In the last fifteen years it has been repeatedly demonstrated that the distribution of sodium and potassium between intracellular and extracellular fluids in living tissues is controlled by the metabolic activity of the cells themselves.^{1, 2} Dr. Renkin and I, working independently, have been interested in the effect of hypothermia upon these cation distributions and are going to discuss some aspects of this phase of the problem of hypothermia. Each of us will present some of his experimental material which is pertinent.

The fundamental phenomena in which we are interested can be summarized briefly. In almost all living tissues, sodium and potassium, the two most abundant tissue cations, are distributed in a characteristic fashion, potassium being the predominant intracellular cation and sodium being its counterpart outside the cell. This separation is not accomplished by the interposition of a barrier (the cell membrane) which is impermeable to the cations. On the contrary, studies with radioactive tracers show that interchange of these and presumably other ions across this membrane is taking place constantly. This implies the expenditure of energy for maintaining the concentration gradients, a concept borne out empirically by many experiments which show that interference with the metabolism of the cell results in disturbance of the electrolyte distributions.

Subjecting tissues to hypothermia is one way of interfering with the metabolism of the cells and for a variety of tissues from homeothermic animals, it has been shown that low temperatures will induce escape of potassium from and entrance of sodium into the intracellular phase, in the direction of thermodynamic equilibrium. Cultured embryonal tissues from chickens,³ guinea pig retina and brain slices,⁴ human erythrocytes,⁵ and hemidiaphragms from rats, have all been shown to lose intracellular potassium when incubated at low temperatures under conditions which in other respects (except for the temperature) are identical with those permitting incubation for several hours or even days at 37° C. without disturbance of intracellular potassium concentration.

It may be remarked here that most of the studies cited, and the experiments which I shall describe, have been directed for technical reasons at potassium, the intracellular cation, and that for the purposes of my discussion there are adequate reasons to assume an opposite movement of sodium even in the experiments in which sodium has not been actually measured. That is, net loss of potassium from the intracellular space may justifiably be considered to be accompanied by simultaneous entrance of sodium into the cell.

Is there a relation between the loss of potassium from these tissues under conditions of hypothermia and the lethal effects of hypothermia in the intact homeother-

* The experiments reported in this paper were performed under contract with the Department of the Army.

mic animal? Of the experiments cited, only those of Brues with chick embryonal tissue, and of Raker, *et al.*, with human erythrocytes have provided an indication of the temperature below which loss of tissue potassium occurred. In both cases the temperature was about 15°. The coincidence of this figure with the body temperature incompatible with survival in artificially chilled warm blooded animals suggested a possible relation between these electrolyte shifts and the lethal effects of hypothermia and led to the experiments which I shall describe.

In the first experiments, hemidiaphragms from rats and from hamsters were incubated in Krebs-Ringer-bicarbonate solutions containing glucose. Water baths set at various temperatures from 38° to 5° centigrade provided temperature control. Previous experiments by Calkins, Taylor, and Hastings had shown (for hemidiaphragms from rats) that incubation at 38° for several hours resulted in no net change in potassium within the tissue, while at temperatures around 5° centigrade, loss of potassium did occur. The aim of the present studies was to determine precisely the temperature below which potassium escaped from the striated diaphragmatic musculature of these two species. The over-all results are tabulated in table I. The critical temperature for escape of potassium from the rat's diaphragm is seen to lie between 15° and 17° centigrade, while for the diaphragms of hamsters the critical range is significantly lower, and under the conditions of these experiments somewhat broader than for the rat's tissue.

These results are particularly interesting in view of the lethal lower limits for body temperature as reported by Adolph.⁷ He found that for the intact rat this value was approximately 15° and for the intact hamster approximately 5° centigrade. Table II tabulates these values comparatively. The correlation is good and suggests that the processes permitting the maintenance of a high concentration of potassium within the cell may be the ones that fail as a result of hypothermia, leading to death.

Hamsters were chosen in this study for comparison with rats because hamsters are hibernators and can undergo spontaneous, periodic and reversible lowering of the body temperatures to levels between 2° and 5° centigrade, a degree of hypothermia in comparison to which the present levels of clinical hypothermia seem sub-tropical.

The next experiments deal with the effects of temperature upon the behavior of the rat's heart. In these experiments, the heart was removed from an etherized, heparinized rat, the aorta was cannulated with a glass tube and placed in a perfusion apparatus, which was inserted in a water bath, the temperature of which could be varied from 5° to 38° centigrade. By way of the aortic cannula, the coronary system of the animal was perfused with Krebs-Ringer-bicarbonate solution containing glucose. The perfusion fluid was collected as it dripped from the heart, was re-oxygenated at the proper carbon dioxide tension, and recirculated at constant pressure through the coronary system. A known volume of perfusion fluid was used—usually between 25 and 50 ml.—and changes in electrolyte composition of this fluid during perfusion yielded information about changes in electrolyte composition of the heart muscle. With the technique, spontaneous, regular contractions of the heart began as soon as perfusion was started, and, with the temperature at 38°, would continue for from two to four hours. Electrocardiograms were taken

TABLE I

Temperature centigrade	Rate of loss of tissue potassium micro Eq./gram wet weight/hour	
	Rats	Ham-sters
37°	-0.20	0.00
	-0.13	0.13
	0.00	0.13
	0.19	0.15
	0.19	0.24
	0.20	0.27
		0.43
17.2°	-0.13	—
	-0.07	—
	-0.03	—
	0.10	—
15.3°	1.17	0.17
15.2°	6.22	—
	4.20	—
	2.45	—
	2.45	—
	2.10	—
	2.05	—
10°	5.94	0.30
	3.00	0.20
	2.53	0.00
	2.43	—
8.5°	—	1.23
6.3°	—	2.00
5.8°	—	3.82
5.6°	—	3.54
5.5°	—	1.26
4.5°	4.84	—
4.0°	5.61	3.53
		3.03

TABLE II

	Minimum survivable body temperature— Adolph ⁷	Temperature below which net potassium loss occurs
Rat	14.8°	15°-10°
Hamster	3°-3.8°	6°-10°

during incubation with one electrode at the dependent tip of the apex of the heart and the other at the base of the aorta. In a typical experiment, perfusion was begun and the heart was mounted in the thermostat. As soon as the beat was regular, the temperature of the bath was slowly lowered, and electrocardiographic tracings were obtained intermittently. From time to time the perfusion medium was sampled for analysis for potassium. After a period of incubation at the lowest desired temperature, rewarming was begun and serial electrocardiographic tracings and medium samples were again obtained.

Figure I shows sample tracings of electrocardiograms from an experiment in which the temperature of the perfused heart was lowered to 8° centigrade and main-

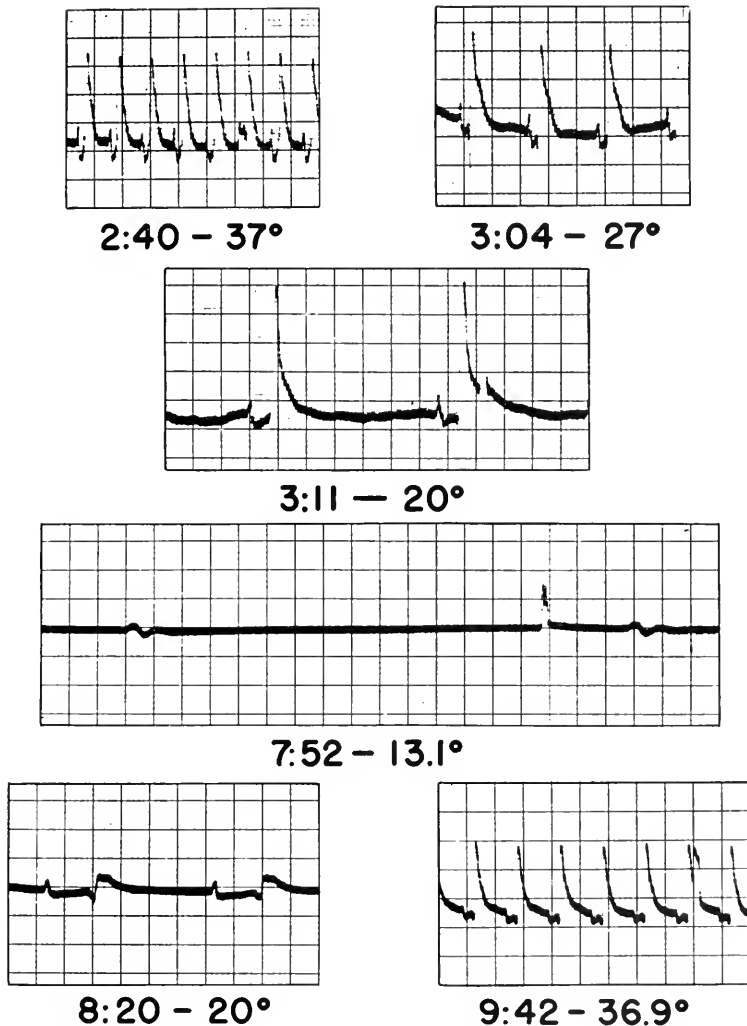


FIG. 1.—Sample tracings of electrocardiograms during reduction of temperature of perfused heart from normal to 8° C. and during rewarming.

tained there for three and one-half hours. The first tracing is taken with the heart at 37° at 2: 40 p.m., soon after perfusion was begun. At 3: 11 p.m. the temperature had fallen to 20° and a very slow heart rate is noted. At 3: 40 p.m. the temperature was 14.8° and visible and electrical systole had ceased. At 3: 54 p.m. the temperature reached 10° and remained between 8 and 10° until 7: 29 p.m. when warming began. At 7: 52 p.m. a visible beat was noted and simultaneously resumption of electrical systole appeared. At 8: 20 p.m., the temperature was again 20° and a regular beat was recorded. At 9: 42 p.m. the temperature was 36.9° and return to a rate near the original was observed.

Table III summarizes in tabular form the results of experiments with the isolated heart preparation. The notable features are (1) cessation of heart beat between 12° and 15° in all preparations except that of 12-10-54 in which isolated contractions were observed at 8°; (2) the spontaneous resumption of heart beat in all preparations when the temperature returned again to 12° or 15° even in preparations with hypothermic asystole of over eight hours duration; and (3) no significant loss of myocardial potassium to the medium even at low temperatures.

These perfusion experiments show clearly for the rat's heart that hypothermic asystole is completely reversible and are evidence that for this animal, hypothermia is not lethal because of a direct effect upon the myocardium. Perhaps the most important difference between the perfused preparation and the heart in the intact hypothermic animal is that in the former, perfusion and oxygenation of the coronary circulation is independent of the contraction of the ventricles, while in the latter, myocardial anoxia must ensue as soon as the pumping action of the heart ceases, thus complicating the possibly reversible effects of hypothermia with the known lethal effects of anoxia.

In contrast with diaphragmatic muscle and the other tissues cited at the outset of this paper, the perfused hypothermic heart does not undergo potassium loss. This was an unexpected observation, and corresponds in superficial respects at least to the observations of Dr. Renkin on the perfused hind leg, of which you will hear

TABLE III

A: Hypothermia

Experiment	Temp. (C.) at which beat ceased	Temp. (C.) at which beat returned	Duration of asystole	Lowest temp. (C.)	% initial tissue K ⁺ in tissue at time of rearming
11-15-54	15.2°	13.1°	4 hrs. 21 min.	8°	92%
12-10-54	8.3°	13°	7 hrs. 56 min.	7°	89%
12-14-54	12.2°	12.4°	4 hrs. 20 min.	7.8°	87%

B: Control tissue at 37° throughout incubation

(Experiment of 11-10-54)

Sample	Time	% of initial tissue K ⁺ remaining
1	0	100%
2	1 hr. 2 min.	90.4%
3	2 hrs. 23 min.	75.5%
4	2 hrs. 52 min.	75.1%
5	3 hrs. 20 min.	65.8%

shortly. Further evaluation of this phenomenon and the interpretation of its significance must await studies with radioactive tracers.

Dr. Calkins: I have read Dr. Taylor's paper to you and would like to add a final comment of my own. It seems apparent that despite the experience with the isolated rat and hamster diaphragm, there is reason to doubt that loss of intracellular potassium *per se* has any direct relationship to the cause of death from hypothermia.

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POTASSIUM EXCHANGE IN PERFUSED MAMMALIAN SKELETAL MUSCLE*

E. M. RENKIN

A variety of isolated mammalian cells and tissues immersed in saline fluids have been found to suffer a net loss of potassium ion in the cold. These include red blood cells,^{1, 2} white blood cells³ and skeletal muscles.⁴ Measurements of K^+ exchange rates with radioactive K^{42} indicate that (1) both influx and outflux of K^+ are reduced at low temperatures and (2) influx is reduced to the greater extent, and net loss results. In the case of frog muscles, influx and outflux are equally reduced in the cold, and tissue K^+ balance is maintained at temperatures as low as $0^\circ C$.⁵

Dr. Taylor has found that in the rat's heart *perfused* with a saline medium, K^+ balance is maintained at $8^\circ C$.⁶ My own observations on perfused mammalian skeletal muscle demonstrate that this tissue, when perfused rather than immersed, is also capable of maintaining its intracellular K^+ level in the cold, at temperatures down to $3^\circ C$. Tracer equilibration experiments with K^{42} show that K^+ influx is reduced at low temperatures, and since K^+ balance was maintained, K^+ outflux must be reduced to the same extent.

The experimental procedure was as follows. The hind leg of a cat was amputated at the hip joint and arranged to be perfused through the femoral artery by a mechanical pump-oxygenator. The perfusion fluid was either fresh cat blood diluted with Ringer's solution or a solution of purified hemoglobin in cat plasma. Heparin was used as anticoagulant. The outflow of perfusate from the femoral vein was returned to the perfusion reservoir, thus a fixed volume of fluid, 150 to 200 ml., was recirculated through the tissues. Blood flows were maintained at normal resting levels or higher, 3 to 10 ml./min. per 100 gm. The weight of the perfused preparation was usually between 300 and 400 gm.; 80 per cent of this was skeletal muscle.

The total amount of K^+ in the perfusion fluid was small compared to the amount in the tissues of the hind leg: $4.3 \text{ mEq./L.} \times 0.2 \text{ L.} = 0.86 \text{ mEq.}$ compared to $75. \text{ mEq./kgm.} \times 0.3 \text{ kgm.} = 22.5 \text{ mEq.}$ Consequently, if a small amount of K^+ was lost by the tissues, it produced a large rise in plasma $[K^+]$. Figure 1 illustrates the time-course of plasma $[K^+]$ in typical perfusions at $35^\circ C$. and at $3^\circ C$. At 35° , after remaining constant for an hour, plasma $[K^+]$ rose linearly, indicating net loss from the tissues at 1.5 mEq./hr. or 0.02 per cent tissue K^+ per minute. The reason for net loss at body temperature is not known. It is relatively slight, and does not appear to be related to injury to the tissues during preparation of the hindleg, glucose content of the perfusion fluid, or the presence or absence of red blood cells in the perfusion fluid. The delayed onset of the loss suggests that accumulation of non-volatile metabolites in the perfusion medium may be responsible, since no provision exists for their removal in the perfusion circuit. It is interesting to note, in this connection, that Andres and others⁷ reported net loss of K^+ from the forearm muscles

*The experiments reported here were carried out in the Biology Department, Brookhaven National Laboratory, Upton, New York, under the auspices of the U.S. Atomic Energy Commission.

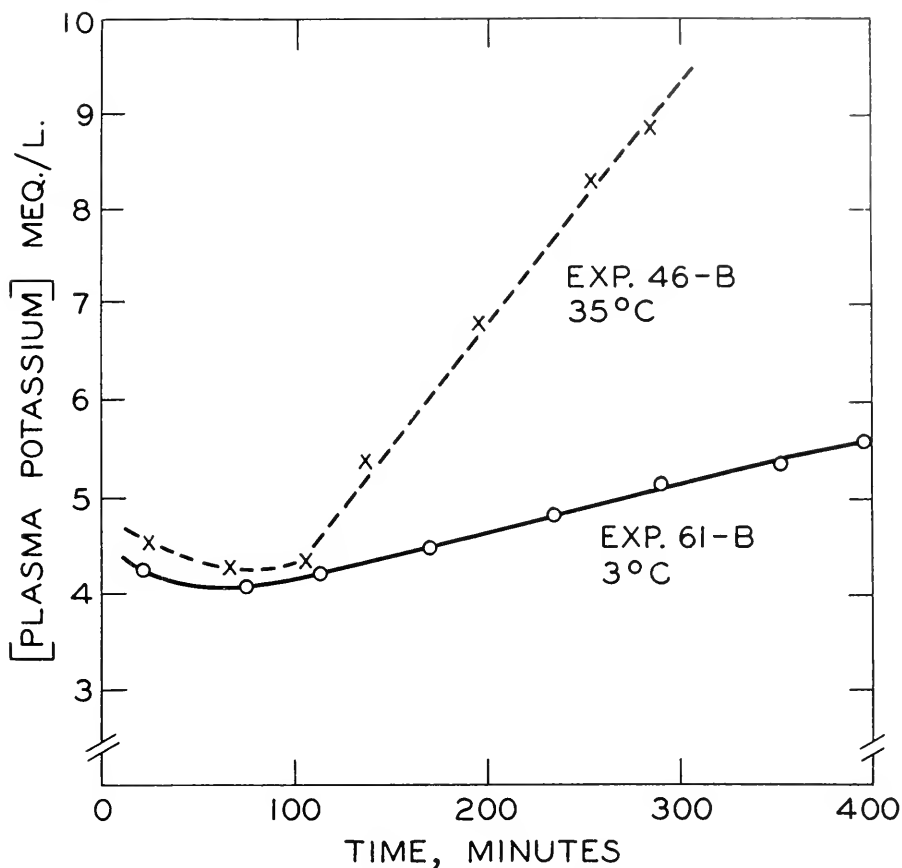


FIG. 1.—Loss of potassium by tissues of the perfused cat hindleg.

of intact, resting man at a comparable rate, 0.005 per cent per minute, and suggested that it may be due to diurnal variations in potassium balance.

At low temperatures, 3° to 9° C., net loss of K^+ was markedly decreased. In the experiment illustrated (fig. 1), the loss was only 0.004 per cent tissue K^+ per minute at 3°. In six hours of perfusion at this temperature, only 1.2 per cent of total tissue K^+ was lost, in contrast to a loss of more than 50 per cent in one hour for rat diaphragm soaked in Krebs' saline at 2° C.⁴

In experiments at normal and at low temperatures, the equilibration of tracer K^{42} between perfusion fluid and perfused tissues was studied. Arterial radioactivity was measured continuously with a recording ratemeter. The experiments could not be run long enough for isotopic equilibrium to be reached, and in calculating equilibration kinetics it was *assumed* that all tissue K^+ is exchangeable. The equilibration curves followed a double-exponential course, one possible interpretation of which is that plasma K^+ equilibrates with two independent compartments of tissue K^+ . Table I has been prepared on this assumption, and lists compartment sizes and calculated "exchange rates" at high and low temperatures. Since only 2 per cent of tissue K^+ is extracellular, both components must represent subdivisions of intra-

TABLE I

APPARENT POTASSIUM EXCHANGE RATES IN THE ISOLATED, PERFUSED HINDLEG OF THE CAT

Tem- perature ° C.	'Fast' compartment		'Slow' compartment	
	% total K ⁺	K ⁺ exch. % min. ⁻¹	% total K ⁺	K ⁺ exch. % min. ⁻¹
35°	20.	0.45	80.	0.016
5°	24.	0.17	76.	0.014

cellular K⁺. The partition of cell K⁺ between the "rapidly exchanging" and "slowly exchanging" compartments is essentially unaffected by temperature. The calculated "exchange rate" between plasma and the "fast" compartment is reduced by slightly more than half over a 30 degree experimental decrease in temperature, a low temperature coefficient indeed, and for the "slow" compartment, there is scarcely any effect of temperature at all.

Before we can interpret the "isotope exchange" data, we must examine what it is that an isotope equilibration experiment measures. Consider a K⁴² ion in the plasma. *First*, it is carried by the blood stream to the capillary bed, *second*, it diffuses across the capillary wall, *third*, it diffuses through the interstitial fluid, and *finally*, it arrives at the muscle cell membrane and is transported across. Thus a chain of transport processes exists, and the slowest member of the chain will determine the overall transport rate. As far as the "slow" compartment is concerned, blood flow appears to be the limiting factor in determining the rate at which K⁴² equilibrates. If we recalculate the K⁺ "exchange rates" as plasma clearances (exchange rate divided by plasma concentration), we find that the slow compartment clearance is the same as the clearances of Na²⁴ and sucrose, two substances which differ greatly in their distribution in the tissues and in their mechanisms of transport. Table II compares plasma clearances of both cations at normal and low temperatures. Both slow compartment clearances are independent of temperature. We conclude therefore, that the slowly equilibrating component for both ions (and also for sucrose) represents a portion of the perfused hindleg which is poorly circulated, mean plasma flow about 0.2 ml./min. per 100 gm., and in which the low rate of blood flow limits the blood-tissue transport of these substances.*

TABLE II

PLASMA CLEARANCES OF POTASSIUM AND SODIUM,
ML./MIN. X 100 GM.

(a) 'Fast' compartment clearances		
Tem- perature ° C.	Na ²⁴	K ⁴²
35°	1.23	1.54
3°-10°	1.44	0.71
(b) 'Slow' compartment clearances		
Tem- perature ° C.	Na ²⁴	K ⁴²
35°	0.19	0.23
3°-10°	0.17	0.21

In the "fast" compartment, which presumably represents the well-perfused remainder of the tissues, the limiting step in the transport chain for K^{12} is not clearly indicated. The low temperature coefficient, Q_{10} about 1.2, suggests that transepillary or interstitial diffusion may be the limiting process. Transport of K^+ across cell membranes by chemical binding to a carrier molecule might be expected to have a Q_{10} of about 2 or 3, characteristic of a chemical process. However, similarity between clearances of Na^{24} and K^{12} at 35° makes it possible that here too, blood flow may be the limiting step. In this event, one of the other processes with a higher temperature coefficient must become rate-limiting at 5° C. Consequently, all we can say about cell membrane exchange of K^+ at low temperatures is that K^+ influx must take place at a rate equal to or exceeding the *minimal* exchange rate listed in table 1, 0.17 per cent tissue K^+ per minute in the rapidly equilibrating compartment. And since net loss of K^+ is about 0.004 per cent per minute, the outflux must be just this much greater than the influx.

Initially we observed that whereas thin strips of mammalian skeletal muscle immersed in a bath of Ringer's fluid lose K^+ rapidly in the cold, perfused mammalian skeletal muscle does not. We now come to the question of why this difference should exist. The answer is entirely unknown at present. The most obvious difference between the two preparations is the distance through which diffusion of oxygen, tissue metabolites and potassium takes place between the fluid medium and the cells. The intercapillary distance in the perfused hindleg muscles is of the order of 50 micra, the maximum diffusion distance is thus 25 micra. The thickness of the rat diaphragm is about 0.4 to 0.5 mm., or 400 to 500 micra, the maximum diffusion path being half this, 200 to 250 micra. However, 250 micra should be thin enough for adequate diffusion of O_2 and CO_2 at 37° C., according to Hill's equations,⁹ and at lower temperatures, since metabolic processes are slowed more than diffusion, conditions should be even more favorable. Nor does it appear that the presence of plasma or protein in the perfusion fluid is responsible for the difference, since Taylor⁶ found no K^+ loss in heart muscle perfused with protein-free media in the cold. Continued study of ion transport in perfused tissues is planned, and it is hoped that it will lead to a solution of this and other problems raised by the experiments reported here.

Assuming that tissues in intact animals behave more like perfused tissues than like soaked tissues, we may expect that hypothermia itself will not result in net loss of potassium from the cells. However, other conditions accompanying clinically induced hypothermia may lead to K^+ loss. It has been shown that some of the changes in myocardial excitability in hypothermia may be prevented or diminished by hyperventilation, and are therefore attributable to respiratory acidosis.^{10, 11} There will certainly be much more said about this in some of the papers to follow, and it seems sufficient to point out here that acute acidosis is known to produce loss of K^+ from cells.^{12, 13}

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ELECTROLYTE TRANSFER DURING HYPOTHERMIA

FRANK GOLLAN

I am in the enviable position of being able to confirm in the intact animal the results obtained on isolated organs.

In collaboration with Drs. Normal S. Olsen and Guilford Rudolph we studied electrolyte transfer during hypothermia at the Veterans Hospital in Nashville, Tennessee. Although we also have used tracer techniques, our approach was different from the one of the previous speakers. We avoided asking difficult questions like: how fast does an electrolyte travel to another site in the body, or the even more difficult question of how it gets there. Thus, in a sense, we have given up before we started since we do not believe that our body consists of two or more compartments divided by a semipermeable membrane. Electrolyte transfer depends on such factors as electrostatic charges, hemodynamics, gas exchange, pH, enzymes, and steroid hormones. Therefore, we have asked one simple question only: how much of the injected tracer can be found in a particular tissue after complete equilibration has taken place in the intact body. Thus, we have investigated final concentration instead of turnover rate or mechanism of transfer.

Potassium,⁴² sodium,²⁴ and bromine⁸² were injected into 20 dogs for each isotope and after 18 hours they were anesthetized with thiobarbital. Half of each group were cooled by ice immersion to 23° C. Both groups were artificially overventilated to exclude the influence of anoxic anoxia and acidosis. Then a thoracotomy was performed and samples of blood, resting skeletal muscle, and beating heart auricle were taken.

The results (fig. 1) show that the major changes do not take place in the plasma

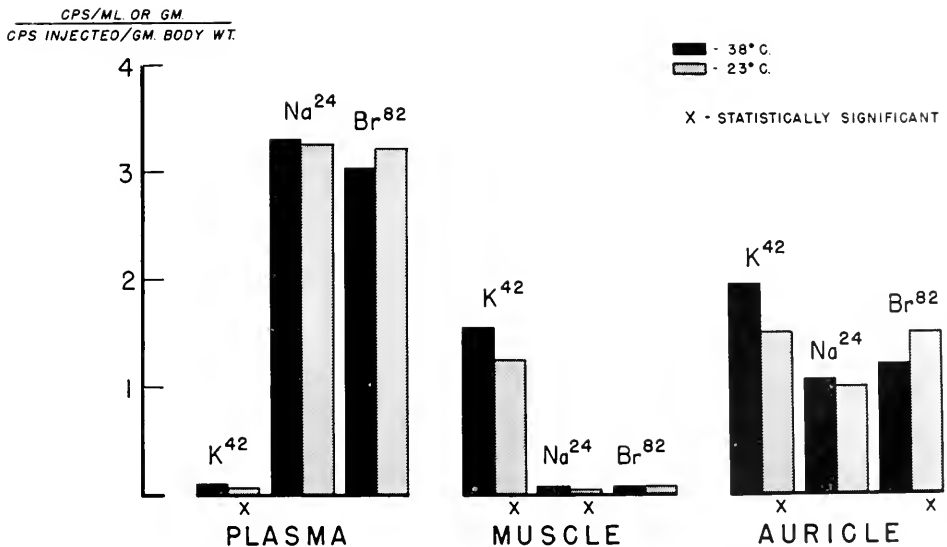


FIG. 1.—Concentration of isotope tracers in plasma, resting skeletal muscle and beating heart auricle.

but in the tissue. The resting skeletal muscle has lost some potassium and maintained its sodium and bromine concentration, whereas the working heart muscle has given up even more potassium and has taken up some bromine.

Because of the increased ventricular irritability in hypothermia and in anoxia normo- and hypothermic animals were subjected to acute anoxia by stopping the respirator for three minutes. After this period blood and skeletal muscle samples were taken again and the other auricle was amputated. The results (fig. 2) indicate that the change from good oxygenation (dark column) to hypoxia (light column) does not alter the plasma concentration of sodium and bromine in normothermic (dotted line) and hypothermic (solid line) animals, but is accompanied by a slight rise in potassium. The electrolyte concentration of the skeletal muscle at both temperatures is not affected by anoxia, except for a slight rise in bromine content at normal temperature. In the heart muscle, however, anoxia causes a marked loss of potassium in a normothermic and a less pronounced loss of potassium in the hypothermic dog. This loss of potassium from the heart muscle during anoxia is accompanied by an increase in sodium content in the normothermic animal only.

The changes of intra- and extracellular electrolyte transfer can be shown more distinctly by establishing a potassium to sodium ratio (fig. 3). During hypothermia the skeletal muscle is able to maintain this ratio fairly well, whereas the heart muscle undergoes a reduction of the K/Na ratio. During anoxia at normal body temperature the K/Na ratio of the skeletal muscle is decreased, but is maintained during hypothermia. The same phenomenon in greatly exaggerated form occurs in the heart muscle where anoxia causes a severe shift of the K/Na ratio in favor of sodium at normal body temperature and a slighter shift during hypothermia.

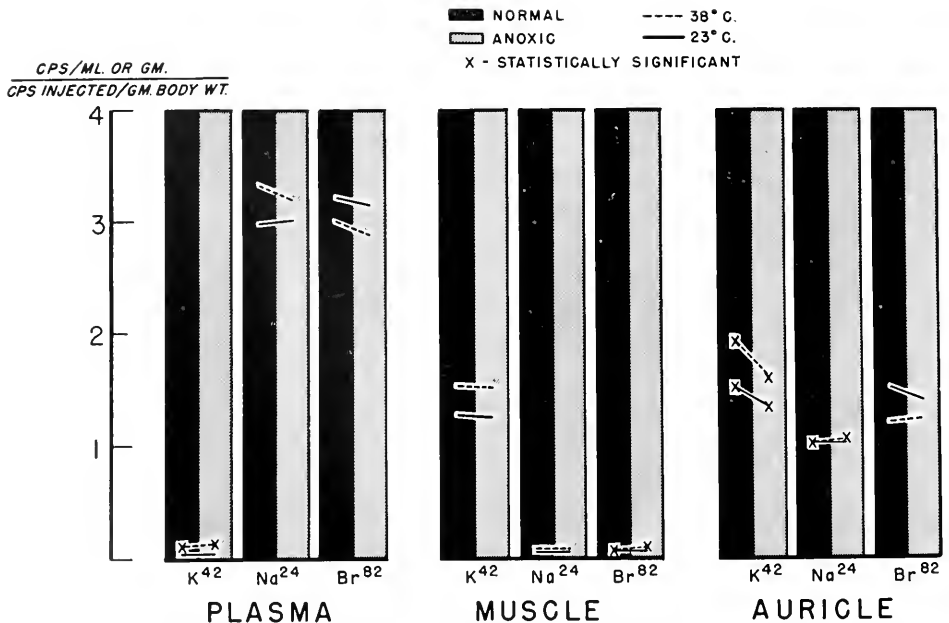


FIG. 2.—Changes in concentrations of K, Na and Br from normal oxygenation to anoxia.

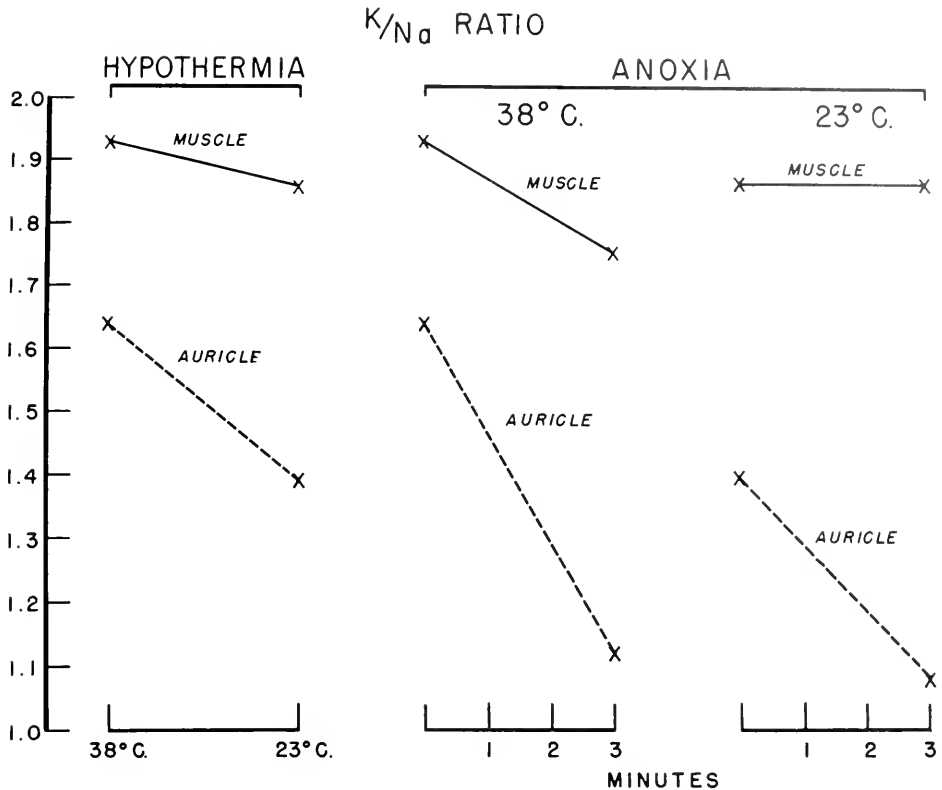


FIG. 3.—Changes in K/Na ratio during hypothermia with normal oxygenation and during anoxia at normal and hypothermic temperatures.

Thus, the increased excitability of the myocardium in well oxygenated hypothermic dogs and in animals subjected to anoxia has a common chemical denominator in the decreased K/Na ratio.

The inability of anoxic tissue to retain its potassium and to repel sodium is well documented in the literature. The same, though much milder, disturbance in the hypothermic myocardium can be interpreted as the result of the prolongation of the activity phase with increased potassium permeability during the long Q-T interval, or a kind of Fenn effect in slow motion.

It is not too difficult to understand that if all the other organs in hypothermia "go to sleep," the heart still has to stay awake and to go on with its work. Although the passengers in the cabins of the ship are all asleep, the fellows down in the boiler room still have to shovel the coal, and they lose potassium while lifting their load. They do not shovel the coal as fast as when their temperature was high, but now each single move takes much longer and they are not given enough time off to recover from their potassium loss.

The increased solubility of oxygen in water and plasma at temperatures close to 0° C. when oxygen dissociation from hemoglobin is negligible, can assume biological importance in large nonhibernating mammals. If the blood of dogs is circulated,

oxygenated, and refrigerated in a small pump-oxygenator, a degree of over-oxygenation can be achieved in which the arterio-venous difference in oxygen and carbon dioxide content almost disappears (fig. 4). At the lowest temperature of 3° C., measured in the right heart, we were then dealing with animals practically devoid of venous blood. The oxygen consumption of such dogs in respiratory and cardiac arrest can be calculated from the arterio-venous oxygen difference in the inlet and outflow of the pump-oxygenator. The oxygen consumption below 10° C. (fig. 5) was exactly where one would expect it to be, namely on a line between the known value at 16° C. and 0° C. Thus, at 5° C. the oxygen consumption of a dog is $\frac{1}{2}$ of normal. Since the blood flow of about 45 cc./kg./min. exceeded by far the necessary flow of about 7 to 10 cc./kg./min. to cover the needs of such animals, the total amount of oxygen supplied (closed circles) would have been sufficient to keep dogs at about 30° C. in oxygen balance. The content of physically dissolved oxygen in plasma rose from 0.3 volume per cent at normal body temperature to about 4.0 volume per cent at 5° C. and therefore even the amount of dissolved oxygen supplied (open circles) exceeded the oxygen consumption at 5° C.

To test this point we washed out all red cells and substituted Ringer's solution and plasma for blood until the dog's hematocrit was lowered to 0.5 per cent. Before rewarming the circulating fluid the red cells were returned to the circulation and these animals, just like the ones who had the benefit of hemoglobin, survived and did not show any sequelae of this trying procedure.

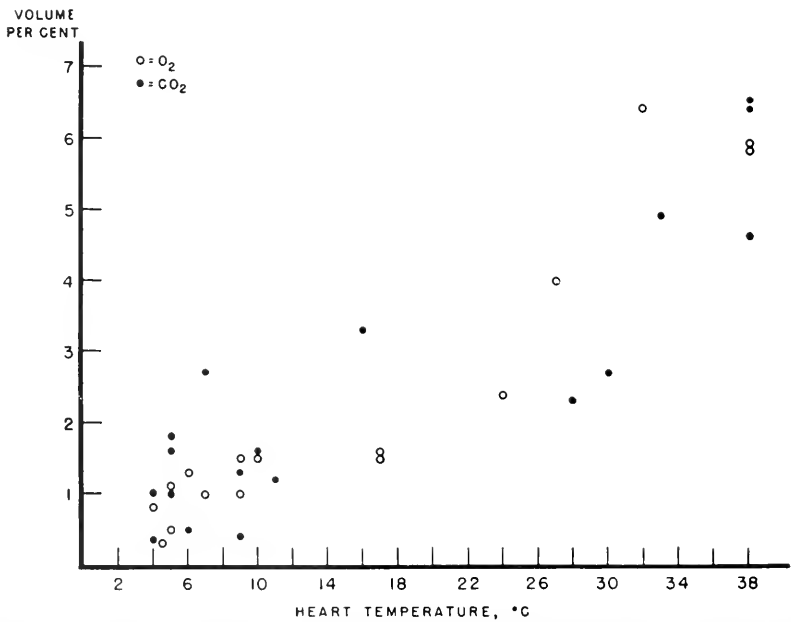


FIG. 4.—Arterio-venous difference in O₂ and CO₂ content during hypothermia by blood cooling.

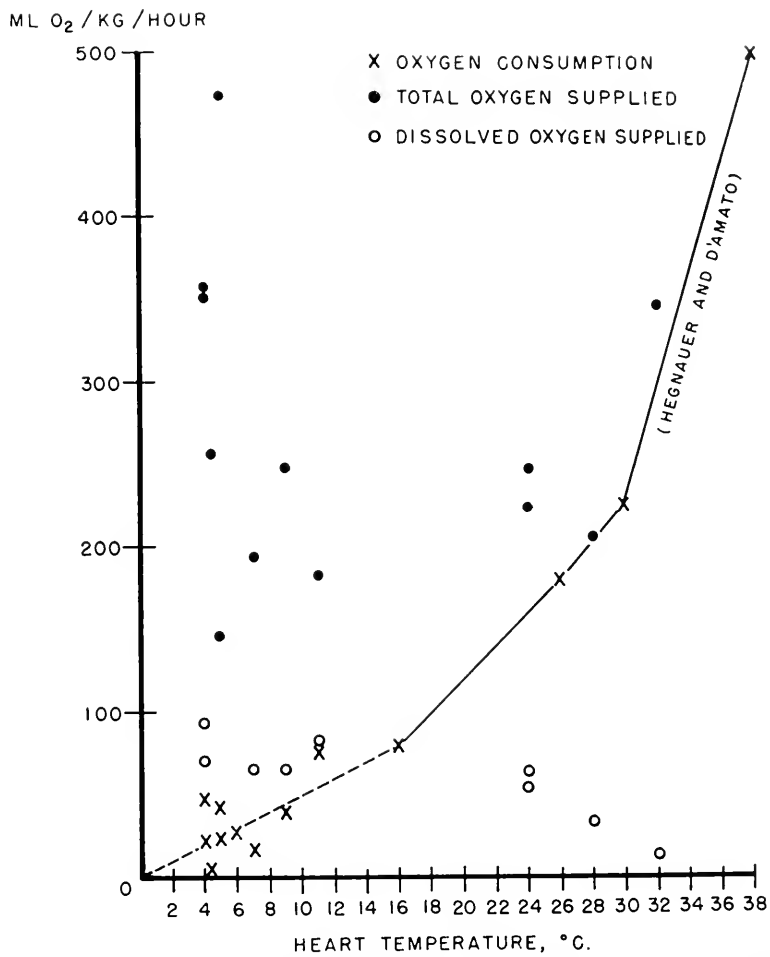


FIG. 5.—Oxygen consumption and oxygen supply in severe hypothermia with pump-oxygenator.

MYOCARDIAL BALANCE OF POTASSIUM

HENRY SWAN

We have tried to study the problem of the myocardial balance of potassium in the intact animal. In order to do this we have attempted to collect the coronary outflow by catheters passed down through one of the jugular veins to the coronary sinus and securely tied by a suture. The catheter continues into the jugular vein, so during most of the experiment the circulation is intact. A side arm is provided so at any time the flow of the coronary sinus blood can be measured by draining it into a basin and timing it with a stop watch.

After the experiment the animal is killed and the heart weighed. We assume approximately 46 per cent of the heart weight is left ventricle. The estimate that 70 per cent of the arterial flow of the left ventricle comes out the coronary sinus then allows us to compute volume in terms of flow per gram of tissue per unit of time.

Our results parallel those of Dr. Taylor and Dr. Gollan. In the perfused heart, in contrast to the perfused skeletal muscle, there was very little loss of potassium with cooling.

We find under these conditions that the potassium balance of the heart is positive throughout the experiment (Table I). In this experiment the animals are cooled to 30° C., and there is *no* support of respiration. In our laboratory, the respiration slows and we consistently have a fall in pH under these conditions as respiratory acidosis develops.

There is no parallelism in our data, however, between the pH and the positive myocardial balance in the intact animal. As seen in the second line, in the control warm situation, the animal appears to be in very slight positive potassium balance in so far as the myocardium is concerned. On first exposure to 30° C., the positive potassium balance increases, and it is still raised one hour later. On warming, the animal maintains a positive balance but not as great.

There is a similar positive balance for phosphorus. I present figure 1 merely to show that the animal appears to have a positive balance of both potassium and phosphorus at about the same rate at all of the temperatures. These data are means of eight animals. In our laboratory, at 30° C., the mildly acidotic dog shows positive balance of potassium in the myocardium.

TABLE I
MYOCARDIAL BALANCES
(Means and standard deviation of 8 dogs)

	Control	30° C.	One hour later	36° C.	Three hours later
Arterial pH	7.37±.06	7.28±.05	7.23±.09	7.33±.11	7.36±.08
K (μEq./Min./100 gm.)	2.82±1.98	8.04±2.56	5.07±1.76	3.82±1.28	6.13±2.34
P (μEq. Min./100 gm.)	0.70±1.68	3.64±1.08	3.08±1.13	5.88±2.35	3.70±2.75

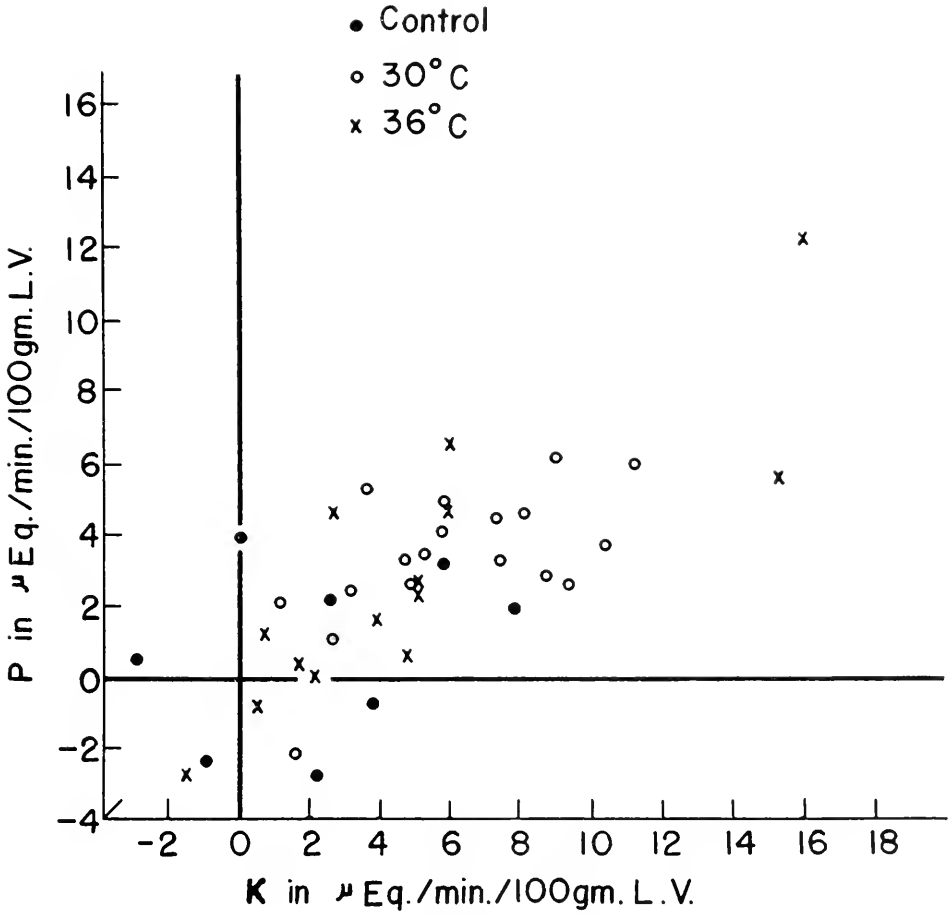


FIG. 1.

EFFECTS OF LOW BODY TEMPERATURE ON TISSUE OXYGEN UTILIZATION

E. F. ADOLPH

Low temperatures have two recognized physiological effects: to kill and to prolong life. A task of many investigators is to define the circumstances under which each effect occurs. We also want to identify physiological processes concerned in each effect.

I shall consider only deep hypothermia, in which body temperatures stand within a few degrees of lethal temperature. For all non-hibernating mammals, the lethal temperature, from which they can not recover without assistance, is well above their freezing points. But in the same range of temperatures, processes are slowed to the extent that survival without an oxygen supply is much prolonged.

Lethal effects of low temperatures have been attributed to many tissues and processes. I shall consider only one of these attributions, namely, that cold death is generally due to lack of oxygen somewhere. I do not know how ancient this theory may be. During World War II it was promoted by Lutz, Werz, and others. I shall

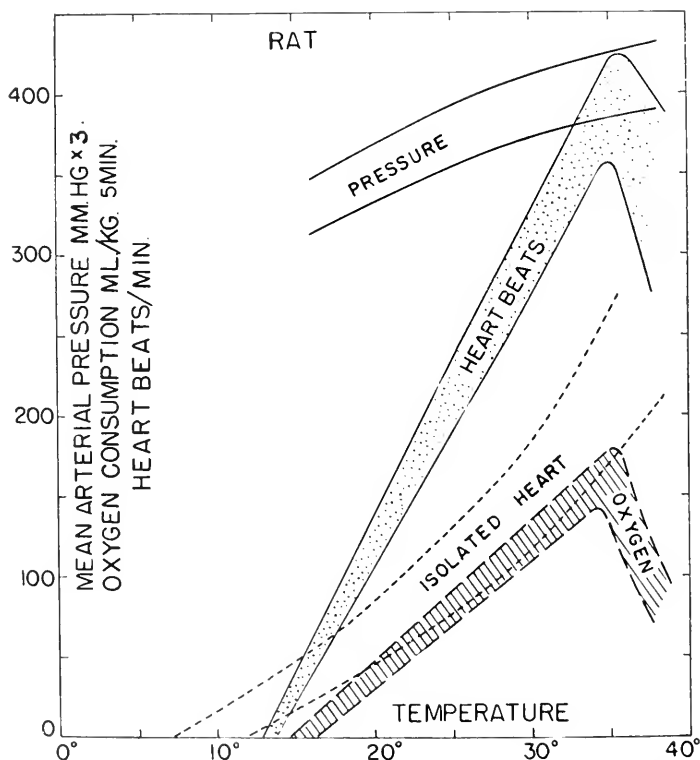


Fig. 1. Modifications in cardiorespiratory functions with body temperatures in unanesthetized rats. Each band includes massed data ± 1 standard deviation. Pressures from Hansen; other data from Adolph, 1951.

conclude that this theory of cold death has almost no evidence in its favor and has some evidence against it. But it will serve the purpose of focusing our discussion.

Delivery of oxygen from the atmosphere to the intracellular enzymes that trade it for electrons can be subdivided into processes of (a) breathing, (b) circulation of blood, (c) transport in blood, (d) oxygen pressure in tissues, and (e) oxygen transfer in cells. I will mention each of these processes in turn.

Breathing. Oxygen consumption in unanesthetized animals such as rats (fig. 1) augments when body cooling begins. As soon as the core temperature drops below 33° , however, the consumption diminishes. It is linearly related to core temperature, becoming zero at approximately the temperature at which breathing ceases, which is 15° for the rat (Adolph, 1950). Just above this temperature the pulmonary ventilation is very slow; nevertheless, in species tested (guinea pig, Gosselin; dog, Rosenham and Penrod; man, Dill and Forbes) it is found to be more than adequate to deliver oxygen to the lung alveoli, as indicated by high pO_2 and low pCO_2 in arterial blood. Therefore, it can be considered that breathing does not limit the mammal's supply of oxygen until it ceases through paralysis by cold. Even then artificial breathing alone does not allow animals to survive much lower body temperatures, and specifically does not, I infer, augment the oxygen consumption at 15° to 20° C.

Circulation. The cardiac output of unanesthetized rats has been measured by dye solution in this laboratory. It decreases linearly with body temperature (fig. 2). The stroke volume of the heart is as large at 18° as it is at 38° , thus having a temperature coefficient of 1. At each temperature the oxygen consumption may be divided by the cardiac output to give the utilization fraction; this fraction does not increase at low temperatures. Evidently the blood delivers adequate oxygen to the

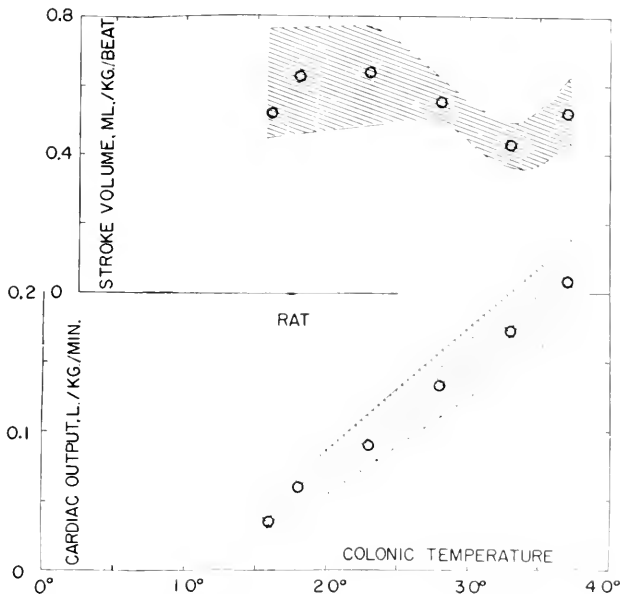


FIG. 2.—Cardiac outputs and stroke volumes of hypothermic rats. New data of R. W. Bullard.

average tissue. Cardiac outputs do not indicate circulatory failure, and arterial pressure is maintained in the absence of chemical anesthesia.

Blood flows to specific organs need also to be considered. If flow should be deficient in a critical area, the sufficient cardiac output would not avail. In anesthetized hypothermic dogs the coronary venous blood was shown (Penrod, Berne) to contain available oxygen. The cerebral blood flow was found (Rosomoff and Holaday) to be as adequate as in normothermia.

Microscopic observations of minute vessels in rat mesentery at 20° and in hamster cheek pouch at 10° reveal that blood ceases to flow in at least half the capillaries and venules without much visible constriction of those blood vessels (this laboratory). These stoppages of flow reverse during rewarming. Therefore, general blood flow in hypothermia diminishes partly by means of total cessation of flow through selected capillaries and venules. Since the cessation is spotty for any given area of mesentery, over-all oxygen supply may remain adequate.

Oxygen transport in blood. When blood is exposed to low temperatures *in vitro* the oxygen dissociation curve (fig. 3) moves far to the left (Brown and Hill, Penrod). This means that oxygen easily combines with the blood's hemoglobin, but dissociates only at unusually low tissue pressures of oxygen (pO_2). Whether cold tissues can operate when oxygen is supplied by the blood at these low pressures depends upon (a) how far the oxygen has to diffuse from blood capillaries to its site, and (b) how low the pO_2 is at which the oxidizing enzymes such as cytochrome oxidase can hand it onward. The first factor is adequate since we find that enough

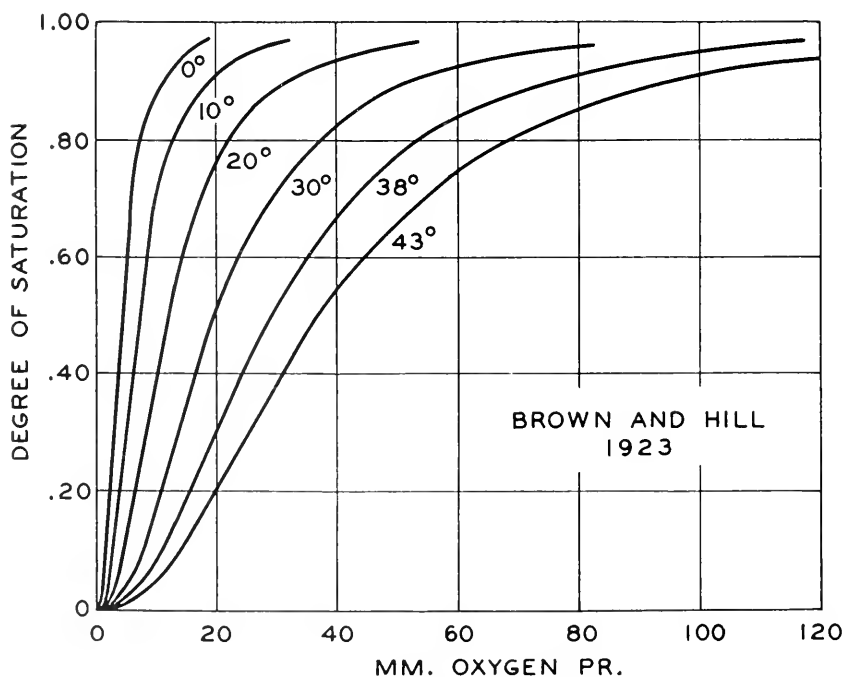


Fig. 3. Oxygen dissociation curves of blood at different temperatures; pCO_2 40 mm. Hg.

capillaries conduct blood in a tissue where it is used. The second factor depends upon the operating characteristics of enzyme systems; they too transfer plenty of oxygen at low temperature.

The over-all delivery of oxygen from the blood to the tissues in general can be estimated from analyses of mixed venous blood. Such analyses in anesthetized dogs (Bigelow *et al.*, Rosenhain and Penrod, Hegnauer) show that the fraction of the blood's oxygen that is unloaded at each circulation is about the same at 18° as at 38°. Cooled tissues accumulate no measurable oxygen debt.

Tissue pO_2 . So far as I am aware, partial pressures of oxygen have rarely been measured by oxygen electrode in any tissue of hypothermic animals (Gollan, 1954). Tissue pO_2 may be estimated by two indirect methods, one based on oxygen content of venous blood, the other based on pO_2 of gas bubbles in body cavities. Hypothermic venous blood *in vivo* shows the diminished pO_2 that would be expected from the shift of oxygen dissociation. Analyses of subcutaneous gas bubbles in rats (this laboratory) also reveals the expected decrease of tissue pO_2 .

Oxygen consumption of isolated tissues likewise diminished greatly (fig. 4). Rat liver (Fuhrman and Field, 1945), brain (Field *et al.*, 1944), heart (Fuhrman *et al.*, 1950), and kidney (Fuhrman and Field, 1942) have been measured. Temperature quotients as well as thermal increments are fairly uniform in all ranges of temperature. The limiting pO_2 of air at which oxygen consumption would decrease in slices of different tissues placed at various temperatures has not been ascertained; this study would tell us whether those tissues *in vivo* can be getting all the oxygen they could use at the pO_2 prevailing there.

Oxygen transfer in cells. Finally, the enzymic transfer of oxygen into carbon

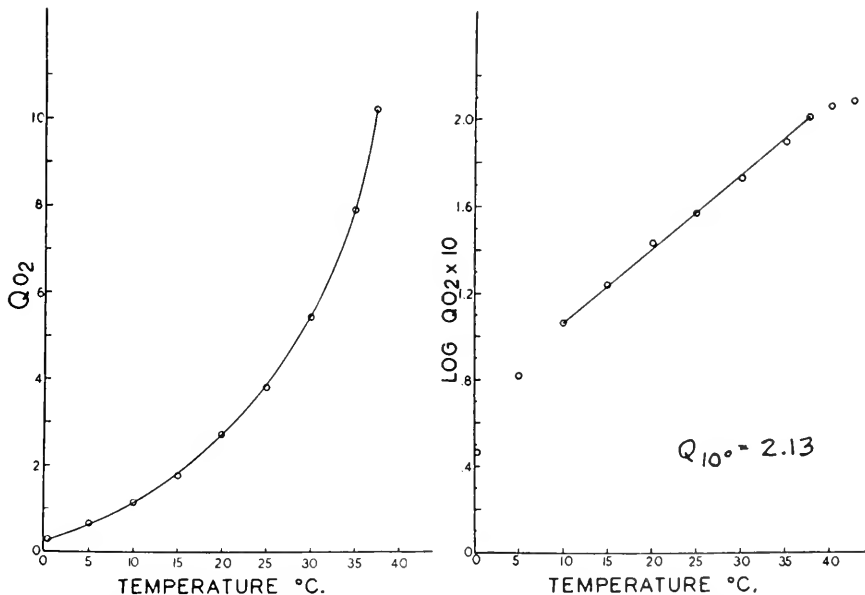


FIG. 4.—Oxygen consumptions (QO_2) at various temperatures of brain slices from rats. From Field *et al.*

dioxide and water may be considered. Is it blocked by low temperatures to an extent one would not expect from the decreased velocities of chemical reactions?

Though slices of isolated tissues consume less oxygen with lower temperatures, they do not suddenly cease consumption at any one low temperature. I interpret this to mean there is no "physiological zero" above freezing at which rat tissues cease to carry on oxidations.

Some tissue homogenates and semi-purified oxidizing enzyme systems have been studied at low temperatures. These also show no evidence of irreversible damage within a few hours at 0° C. in their capacities to transfer oxygen (F. A. Fuhrman *et al.*, 1944). Cytochrome oxidases in particular take up oxygen with ease near 0° C. While it is possible that another reaction, such as phosphorylation, will limit oxygen or electron transfer at low temperature, I conclude that none has been found to do so by the methods so far tried.

Note. Two special processes cease before others when mammals are cooled, namely, breathing and heart-beating. All processes, including these, may recover after an hour or two at 0° (Andjus, Gollan, 1955) whether or not oxygenated blood is being pumped through the tissues concerned. Are these two processes suspended through failure of oxidations? For breathing, no direct answer is available; presumably we should ascertain whether oxygen is still being consumed as demanded in every minute part of the reflex arcs that act in breathing. For cardiac activity the evidence is decisive: oxygen is available and utilized by hearts that have reached standstill or ventricular fibrillation (Hegnauer, Badeer). I conclude that synaptic transmission, breathing, and pumping, the known vital processes that stop at body temperatures above 5° C., probably do not lack oxidative energy. My general experience also suggests that we rarely find under natural conditions a single limiting factor, such as oxygen supply or use, controlling any process, and we would be well advised to study the whole system of equilibria that shift with diminishing temperature.

SUMMARY. We have considered five stages in the delivery of oxygen to the energy-yielding processes upon which cells depend for continuance of their work. In all of these stages, as in oxygen consumption of the whole body, we find evidence of oxygen sufficiency. Reduction of oxygen consumption in deep hypothermia is itself dictated by the lessened demand for oxygen in every metabolizing cell. Cessation of breathing and of heartbeats, upon which oxygen delivery ordinarily depends, itself does not arise from inability of the medulla or the heart to metabolize oxidative energy. I conclude that cold death results from changes other than failure of oxidation. Far from producing anoxia, therefore, hypothermia prolongs the endurance of it.

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OXYGEN CONSUMPTION OF MAMMALIAN TISSUES AT REDUCED TEMPERATURES

F. A. FUHRMAN

The problem of temperature coefficients of isolated tissues, which Dr. Brown and Dr. Horvath touched on, can be summarized in figure 1.

The curve which Dr. Adolph showed was the one relating the oxygen consumption of the brain to temperature over a range of approximately zero to 40° C. I have added to that curve the oxygen consumption of a number of other tissues determined in the same way. These are all slices of tissue studied under as nearly identical conditions as possible in Ringer's phosphate-glucose solution.

In general all of these curves are similar to the one which Dr. Adolph showed for the brain in that they are smooth over most of their course. It is now possible to carry this below zero for the brain and, if freezing does not occur, the curve is still smooth, a continuation of the one shown here.

Temperature coefficients at the various temperatures are not necessarily constant over the whole temperature range, and they are not constant from one tissue to another. The temperature coefficient, Q_{10} , of the brain is approximately constant over a good part of the temperature range (10° to 37.5° C.). For the other tissues this may or may not be so. In general the Q_{10} 's range between 2 and 3 over the central part of the temperature range, from approximately 10° or 15° C. up to about 35° or 37° C. Outside of this range the temperature coefficients may fluctuate widely, in general becoming much larger.

It has been shown by Field and his co-workers that at 37° C. one may obtain a reasonable approximation of metabolic rate of the whole animal by summing the metabolic rate of the individual tissues, in which case if one makes allowances for the contribution of respiratory activity, muscle tension and cardiac activity, one approximates the total metabolic rate of the animal from summing the rates of the tissues.

We have made some preliminary calculations of this kind at 18° C., using for the rat at that temperature the total oxygen consumption obtained by Dr. Adolph, which has been confirmed in a few experiments of our own. At this temperature also the metabolic rate of the hypothermic animal can be largely accounted for as the summated respiration of the individual tissues. Again, this illustrates that there appears to be no interference with oxygen delivery over this range of from approximately zero to 37° C.

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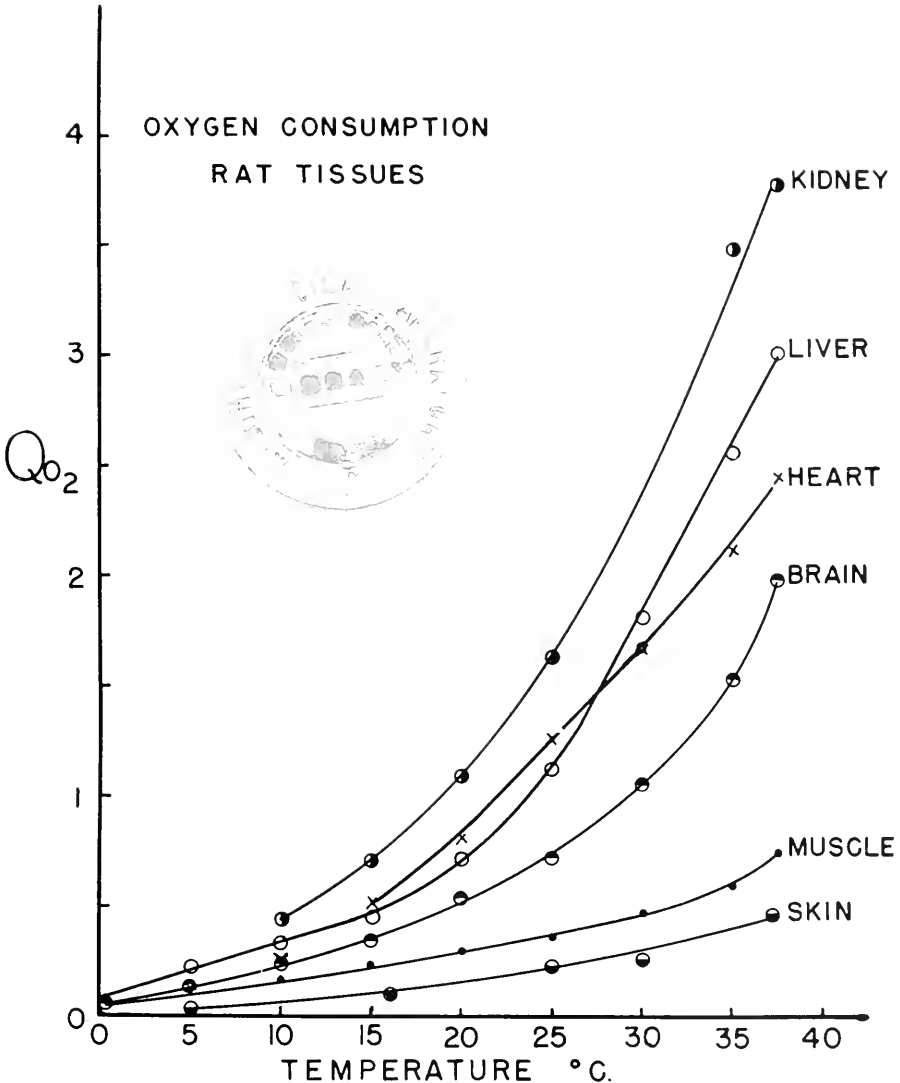


FIG. 1.—Oxygen consumption of rat tissues as a function of temperature. The rate of oxygen consumption (Q_{O_2}) is expressed on a wet weight basis for various tissues of adult albino (Wistar) rats. All tissues were suspended in Ringer-phosphate-glucose medium with 100% oxygen as the gas phase. The sources of data are as follows: Kidney—F. A. Fuhrman and J. Field: *J. Pharmacol. Exper. Therap.* 75:58, 1942; Liver—F. A. Fuhrman and J. Field: *Arch. Biochem.* 6:337, 1945; Heart—G. J. Fuhrman, F. A. Fuhrman, and J. Field: *Amer. J. Physiol.* 163:642, 1950; Brain—J. Field, F. A. Fuhrman, and A. W. Martin: *J. Neurophysiol.* 7:117, 1944; Muscle—N. Hollinger: Ph.D. Dissertation, Stanford University, 1944; Skin—F. A. Fuhrman and G. J. Fuhrman: unpublished.

RESPIRATORY PHYSIOLOGIC STUDIES DURING HYPOTHERMIA

JOHN W. SEVERINGHAUS AND M. STUPFEL

Methods. The major physicochemical effects of changing temperature on blood gas transport mechanisms have been summarized by Dill and Forbes¹ and Rosenhain and Penrod.² Briefly, the solubility of both oxygen and carbon dioxide in blood is increased more than 50 per cent at 20° C. For each degree fall in temperature the serum pK' rises .005 pH units (see below) and the whole blood pH (*in vitro*) rises 0.0147 pH units. The changes in oxygen dissociation curves have been discussed. These changes invalidate the usual nomograms used for computing gas tensions, or pH.

The direct determination of blood gas tensions using the Roughton-Scholander syringe technique will provide the correct tension if the equilibration and absorption procedures are carried out at body temperature. Since it is difficult to adjust baths to exact and rapidly varying body temperatures, it was desirable to be able to apply corrections for small temperature differences. These corrections may be computed from the standard curves for oxygen dissociation, CO₂ solubility, and the known pH and pK' changes. The assumption is made, and has been verified experimentally, that plasma CO₂ content and whole blood oxygen content are not altered by *in vitro* changes in temperature. For oxygen, the fact that temperature alters blood pH, and this in turn alters the dissociation curve in addition to the alteration due to the temperature change at constant pH, must be included. Over small ranges of temperature it is sufficiently accurate to reduce the observed tension, per degree difference between body and equilibration bath, by 6 per cent for oxygen and by 4.7 per cent for CO₂. An accurate expression for these relations is given as follows:

$$P_b = P_o \times 10^{(T_b - T_o) \times f}$$

where P_b and P_o are the gas tensions in mm. Hg at body and water bath temperatures respectively, T_b and T_o. The factor "f" varies with temperature and pH, values being given in Table I for oxygen and carbon dioxide.

These calculated temperature corrections have been subjected to experimental confirmation using the Riley modification of the direct tension technique. The average change for oxygen tension found on reducing blood temperature from 37 to 24° was 54 per cent, which is 6.1 per cent per degree when converted by the above

TABLE I

FACTORS FOR CORRECTING BLOOD OXYGEN AND CARBON DIOXIDE TENSION FROM THE
TEMPERATURE OF MEASUREMENT TO THE TEMPERATURE OF THE SUBJECT

Temp. (C.)	Factors "f" for oxygen				Factors "f" for CO ₂ at all pH values
	at pH 7.0	at pH 7.2	at pH 7.4	at pH 7.6	
37	.02349	.02410	.02475	.02542	.0192
30	.02435	.02496	.02561	.02628	.0200
23	.02523	.02584	.02649	.02716	.0219

formula. (Note: this is not 54 per cent divided by 13°, since the decrease is a fraction of the tension for each increment, hence a logarithmic decrease.) For CO₂, a greater experimental scatter was noted, and the average change observed, 3 per cent per degree, is not considered reliable. There is no reason to doubt the accuracy of the calculated values, based on well established factors.

CO₂ tension was also computed from pH measured on whole blood and plasma CO₂ content, S and pK' using the Henderson-Hasselbalch equation, solved for P_{CO₂},

$$P_{CO_2} = \frac{CO_2 \text{ content}}{S(10^{(pH-pK')} + 1)}$$

S may be obtained by multiplying the values for alpha of CO₂ in water, in standard handbooks, by the figure 0.0544, giving 0.0301 at 38° C.

Determination of pK'. It has been assumed since 1928 that pK' is 6.105 at 38° and 6.19 at 20°, varying in linear fashion between these temperatures. These data are based on an averaging of several investigators' data compiled by Hastings *et al.*,³ and the determination of temperature sensitivity by Cullen *et al.*⁴ To our knowledge, subsequent reports at variance with these have not been incorporated into investigative procedure. Robinson *et al.*⁵ reported a large number of pK' determinations on human sera in 1934, averaging 6.092. Furthermore, if data on horse sera are omitted from Hastings *et al.*, the average is 6.094. With regard to temperature variations in pK, Rossier and Mean⁶ in 1941 reported variations twice as great as those observed by Cullen *et al.* Dill *et al.*⁷ noted changes in pK' with the pH of the serum in which the determination was made, corresponding to a rise of 0.03 for a fall in pH of one unit. With these conflicting data at hand, it seemed wise to determine the pK' variations with temperature and pH, as they would affect our observations. A further reason was that pH standards have been changed through the years, and that most of the data were obtained with hydrogen electrodes, whereas most investigative work is now done with the glass electrode.

Our 41 determinations, on 9 sera, agree well with Robinson, suggesting that pK' is 6.090 at 37.5°, pH 7.4. We found the increase of pK with falling pH to be slightly greater than Dill *et al.*, our slope being .042. At 24° this slope was increased to .062, and the rise of pK' at pH 7.4 agreed with Cullen, a value of -0.005 per degree being found (fig. 1). These data have been incorporated into a nomogram (fig. 2) for the calculation of human serum pK' at various temperatures and pH values.

Physiology of pulmonary function during hypothermia. Hypothermia depresses the spontaneous respiratory exchange. The magnitude of this depression and the temperature of its end point, apnea, depend greatly on the depth of anesthesia, the type and amount of premedication, and individual variations, precluding any valid prediction of these quantitative ventilatory responses to hypothermia. It is usually assumed that assistance to or control of respiration is desirable during hypothermia.

Whenever artificial respiration is used, questions arise concerning its effect on the blood gas tensions, the pH and the circulation. At 37° the problem is the maintenance of normal values. At reduced temperatures an additional problem presents itself: What are the most desirable values of pH and P_{CO₂}, there being no normal? If one holds pH or P_{CO₂} constant, CO₂ is retained. In other species pH may rise

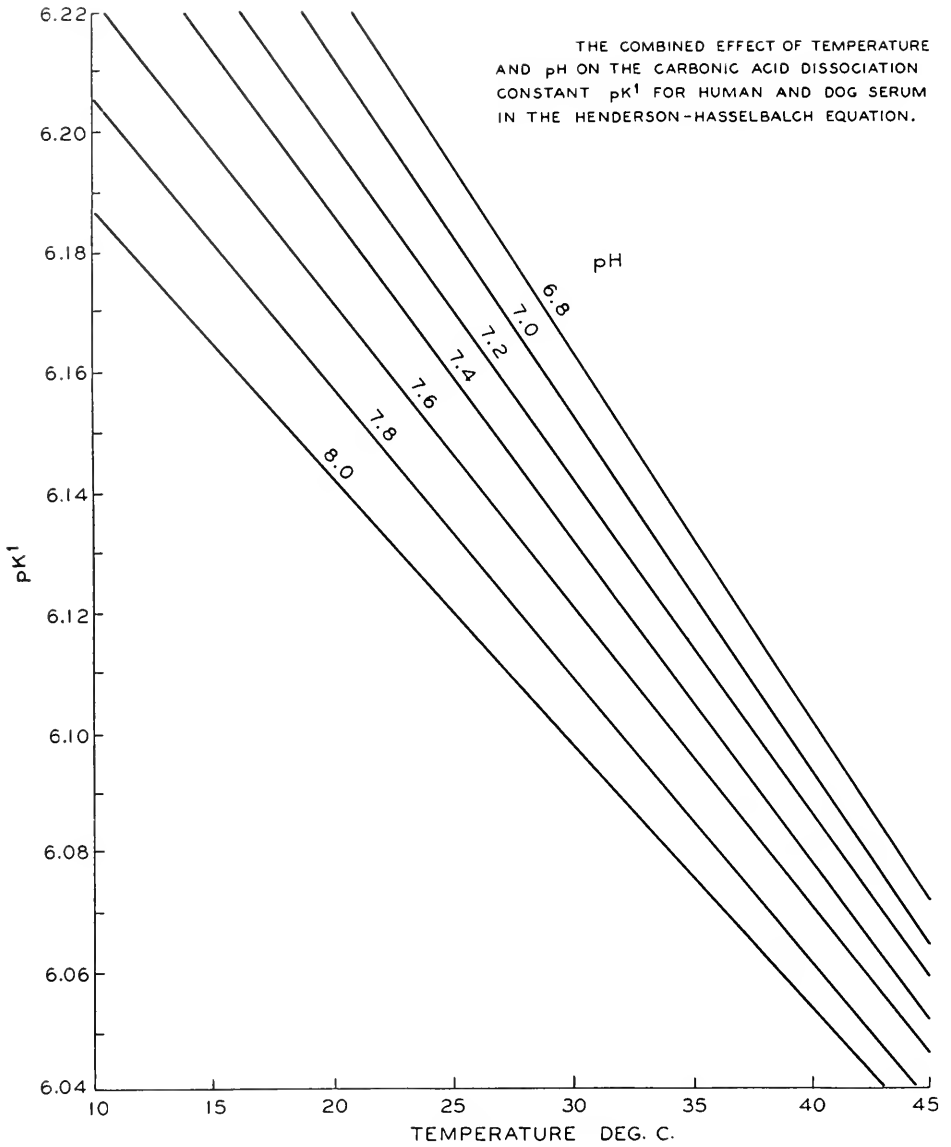
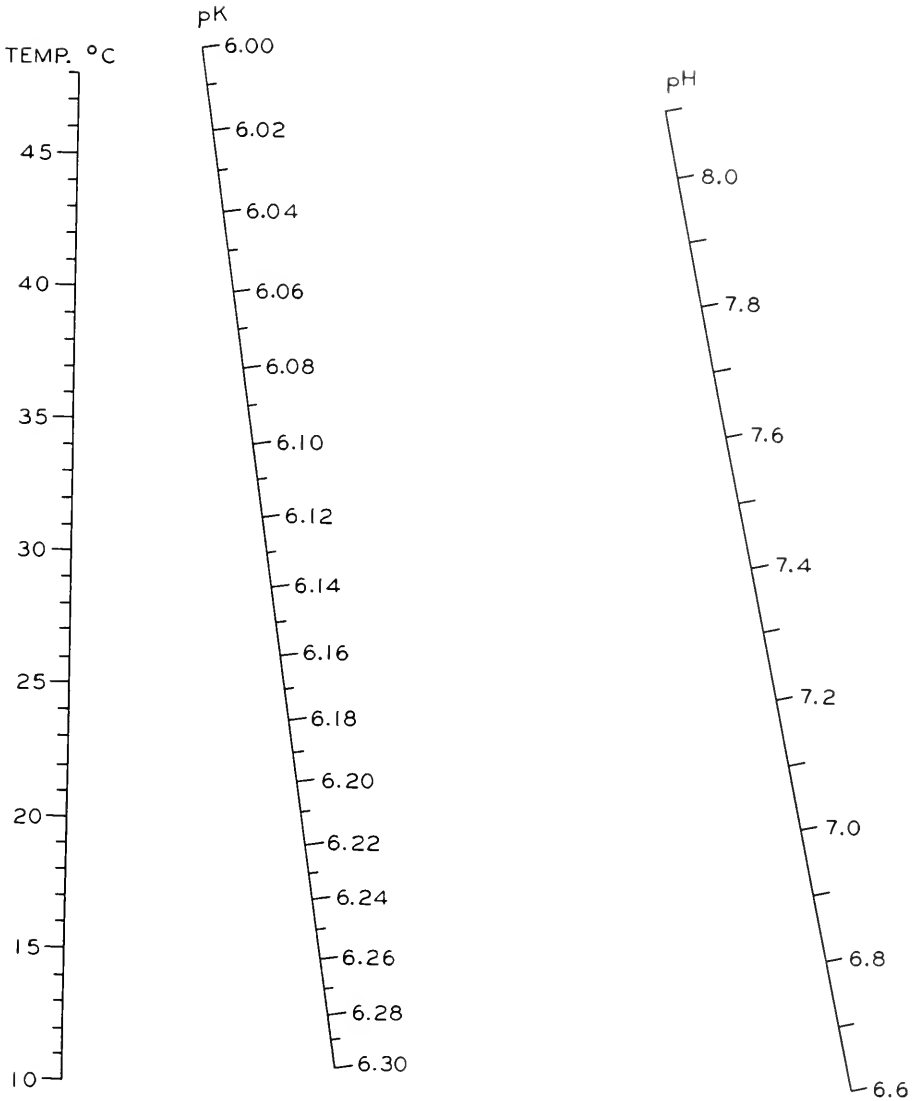


FIG. 1.



NOMOGRAM FOR THE DETERMINATION OF SERUM pK^1 AT VARIOUS TEMPERATURE AND pH VALUES. A STRAIGHT EDGE JOINING THE KNOWN TEMPERATURE AND pH POINTS INDICATES THE PROPER pK^1 FOR USE IN THE HENDERSON-HASSELBALCH EQUATION.

FIG. 2.

during cooling, as in alligators, or fall as in hibernators. The current favoring of hyperventilation is based largely on the incidence of ventricular fibrillation in animals cooled without assisted respiration. On the other hand, vigorous hyperventilation may deprive tissue of oxygen by two factors: (1) the rising pH further shifts the oxygen dissociation curve to low tensions, and (2) cardiac filling and output is reduced.

A further complication arises when an attempt is made to relate ventilation, as measured by tidal volume and respiratory rate, to the resultant pH and P_{CO_2} . At 37° these relationships are conveniently obtained from diagrams. During hypothermia, the metabolic production of CO_2 falls, solubilities change, and the lung may behave differently. Reports have suggested that during hypothermia, CO_2 elimination from the lung may somehow be blocked (Osborn⁸). We have therefore done the following experiments to establish some of the relationships between ventilation and blood gases during hypothermia.

Dogs were curarized, anesthetized and ventilated at known rates with known volumes of air, and cooled in ice packs. Determinations included lung compliance, arterial blood pH and P_{CO_2} , end-expiratory P_{CO_2} , expired air CO_2 concentrations, anatomic and physiologic dead space and alveolar ventilation. Results are as follows:

(1) The anatomic or airway dead space at 25° was increased by 70–90 per cent. A similar increase in the warm animal is obtained with atropine. Bronchoconstriction resulting from vagal stimulation, easily observed as a decreasing anatomic dead space, is blocked at 25°. The cardiodepressor effect of vagal stimulation is greatly depressed or absent at 25°.

(2) Physiologic dead space, calculated from the Bohr formula using arterial P_{CO_2} , is also increased during hypothermia, again due to bronchodilatation.

(3) Alveolar dead space, a portion of the physiologic dead space due to uneven distribution within the lung, is unchanged or reduced during hypothermia. If there were any block in CO_2 excretion, from any cause, it would be expected to enlarge this value.

(4) The difference between arterial and alveolar (end-expiratory) CO_2 tensions is a further measure of uneven distribution, primarily of blood flow. This difference was not altered, or often reduced during hypothermia, again suggesting no impairment in CO_2 excretion.

(5) The slope of the alveolar nitrogen plateau after a single breath of oxygen is a measure of uneven ventilation. No significant change was observed in this slope.

(6) As temperature falls, provided tidal volume and rate are held constant, the arterial P_{CO_2} at 25° falls to about $\frac{1}{2}$ the control value. This fall reflects a comparable decrease in metabolism. The arterial pH usually rises 0.1 unit.

(7) Metabolic acidosis occurs to a variable extent, both at 37° and during cooling, depending on the depth of anesthesia, and the agent used. Acidosis was less severe with pentobarbital than with chloralose.

(8) Compliance tends to decrease during hypothermia, but not significantly more than during a similar span of time at 37°. This progressive fall is probably due to such lung changes as atelectasis, congestion and rarely edema.

In summary, hypothermia leads to an increased anatomic dead space through

bronchodilatation, but no evidence of difficulty in elimination of carbon dioxide has been observed, provided the known changes in blood gas tensions are considered.

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THE GAS TRANSPORT SYSTEM IN HYPOTHERMIA

F. F. KAO

One of the most important aspects of the physiology of hypothermia is the change in the gas transport system which normally is called upon to supply oxygen continuously to and remove carbon dioxide from the metabolizing tissues. During the hypothermic state, the body temperature as well as the oxygen consumption of the organism increases during shivering but decreases when shivering is abolished. The adequacy of the adjustment of the gas transport system during hypothermia can be evaluated by correlating ventilation and cardiac output with oxygen consumption. In dogs, as in man, under normal resting condition, ventilation and cardiac output are precisely regulated to meet metabolic demands, as evidenced by the constancy of the ventilatory equivalent for oxygen (VE_{O_2} , defined as ventilation in liters per minute BTPS divided by oxygen consumption in 100 ml. per minute STPD) and the circulation equivalent for oxygen (CE_{O_2} , defined as cardiac output in liters per minute divided by oxygen consumption in 100 ml. per minute STPD).

During hypothermia, both VE_{O_2} and CE_{O_2} change as a function of oxygen consumption. The relationship between ventilation and oxygen consumption in 12 hypothermic dogs (without shivering) is shown in figure 1, in which ventilation is curvilinearly related to oxygen consumption. The concavity of the curve faces the vertical axis, indicating a decrease in the ventilatory equivalent for oxygen at low levels of oxygen consumption caused by a decrease in body temperature. Figure 2 reveals a similar relationship between ventilation and oxygen consumption in hypothermic

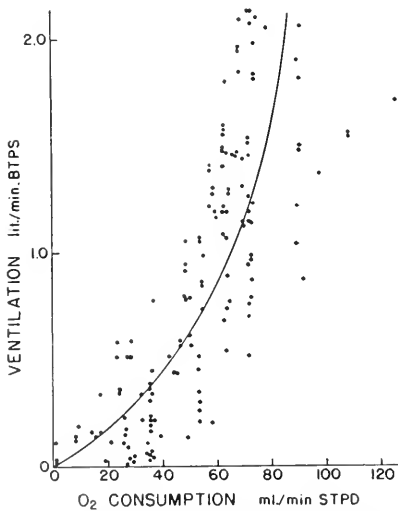


FIG. 1.—The relationship between ventilation and oxygen consumption during hypothermia in dogs (without shivering). For explanation see text.

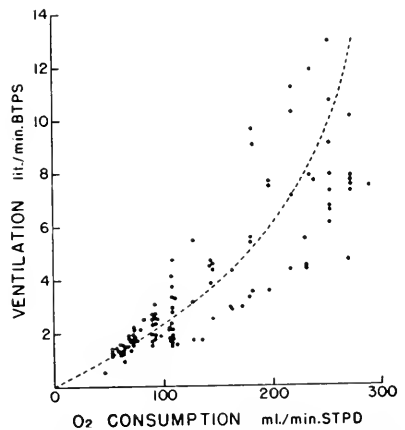


FIG. 2.—The relationship between ventilation and oxygen consumption during hypothermia in dogs with shivering. As shown by the curvilinear relationship between these variables, the ventilatory equivalent for oxygen increases at high oxygen consumption caused by shivering during hypothermia.

dogs, with shivering. An increase in the VE_{O_2} occurs at high levels of oxygen consumption. Since a change in VE_{O_2} indicates change in the alveolar P_{CO_2} it can be predicted that the arterial P_{CO_2} and ventilation must be inversely related in these hypothermic dogs. Figure 3 shows this prediction is true. The dotted line in figure 3 is the predicted result, using the ventilation equation.¹ The solid line in figure 3 represents that fitted to the available data. From these results, it can further be predicted that respiratory acidosis must occur during hypothermia without shivering and respiratory alkalosis exists during hypothermia with shivering. This prediction has been verified by experimental results.²

Cardiac output increases in the hypothermic dogs when shivering occurs, but it decreases in absence of shivering. It is interesting to note that the circulatory equivalent for oxygen is also curvilinearly related to oxygen consumption (fig. 4), indicating that the cardiac output is more than adequate to transport oxygen at low levels of oxygen consumption but less adequate at high levels of oxygen consumption. The significance of this finding as well as of the regulatory process of cardiac output during hypothermia is at present not explained.

During hypothermia in dogs, ventilation fails first, followed by the failure of the heart. The regulation of the gas transport system during hypothermia is of a complex nature because of the numerous stimuli produced during hypothermia, in addition to those prevailing in the normal condition.

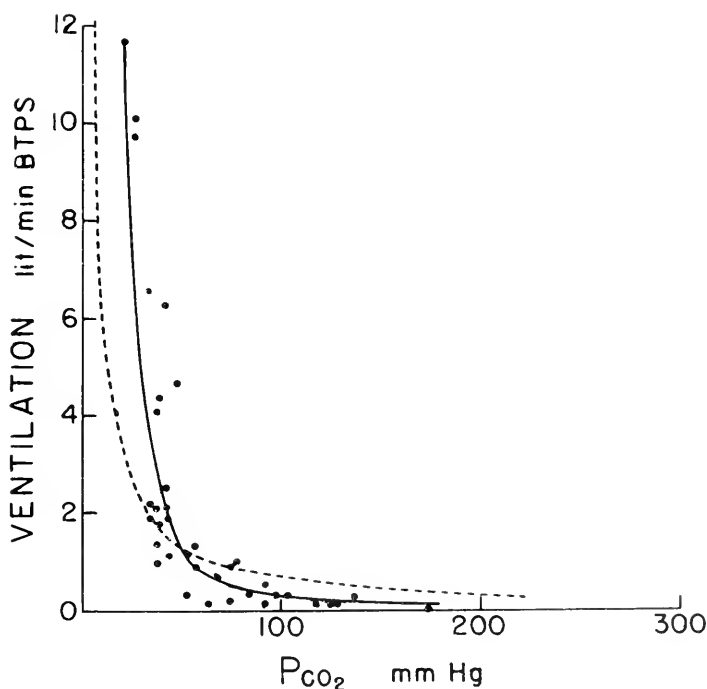


FIG. 3.—The relationship between ventilation and arterial P_{CO_2} . The dotted line represents the predicted results. The solid line is the line fitted to the data obtained during hypothermia, both with and without shivering.

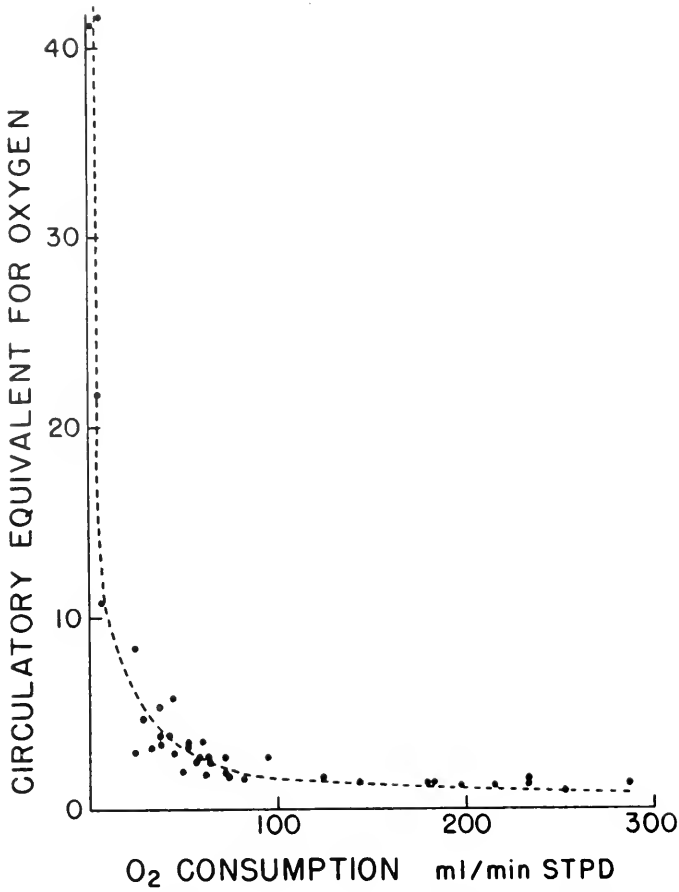


FIG. 4.—The relationship between the circulatory equivalent for oxygen and oxygen consumption in hypothermic dogs both with and without shivering. The fitted line takes the shape of a rectangular hyperbola.

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HYPOTHERMIA IN THE UNANESTHETIZED POIKILOTHERMIC DOG

ALLEN D. KELLER

The reason for attempting to reduce large heat regulating laboratory animals to the completely non-heat regulatory status was threefold. First, it was desired to determine if the ability of the homotherm to maintain body core temperature near constancy was dependent upon nerve cells localized to the hypothalamus as first suggested by Ott¹ on the basis of puncture experiments and later clearly indicated by the brain slicing experiments of Isenschmid and Schnitzler.² Second, if this proved to be so it seemed probable that graded tissue defects in the hypothalamus might permit greater insight into the "whats" and the "hows" of heat regulation. Third, there are many ways in which an ambulatory poikilothermic homotherm could be used as a laboratory "test tube." The effects of thermal variables applied to tissues and organs in their otherwise normal habitat could be determined uncomplicated by superimposed thermal regulatory or anesthesia influences. Tissue chemistry could be "slow motioned" to allow study of changes in intermediary metabolism, blood clotting, antibody formation and similar processes. The thermal spectrum for invading pathogens might be delimited.

The material to follow constitutes a summary-report of progress made toward attaining these objectives.

THE POIKILOTHERMIC DOG

The healthy dog is an exceptionally good heat regulator as judged by its ability to prevent a lowering of its core temperature in the presence of an abrupt and heavy cooling load. This is illustrated in the upper portion of figure 1 by the colonic temperature curves for a dog when, without any previous conditioning to cold, it was abruptly subjected to an ambient temperature of 3° C. for 6 hours and of -20° C. for a 24-hour period. During the 3° C. exposure, deep colonic temperature was maintained at 38° C. for the period of the exposure. When subjected abruptly to -20° C. core temperature rose from 38 to 38.7° C. during the first hour of exposure and remained at this elevated level during the remainder of the 24-hour run. Thus, increasing the cooling load had a sustained elevating effect.

The completely poikilothermic animal. The ability to hold body temperature at or slightly above the homothermic level under such circumstances is due to automatic regulatory processes mediated by nerve cells localized to the gray matter in the hypothalamus.³ This is evidenced by the fact that when the nerve fibers which descend from this area are severed the animals lose all ability to regulate against cold. The colonic temperature curves of two such preparations when subjected to an ambient temperature of 3° C. are shown in the lower portion of figure 1. It was necessary to incubate them at 30° C. to keep the body temperature at the normal 38° range; when they were abruptly placed in an environment of 3° C. the colonic temperature fell progressively in a straight line to reach 28° C. at the end of a 3-hour

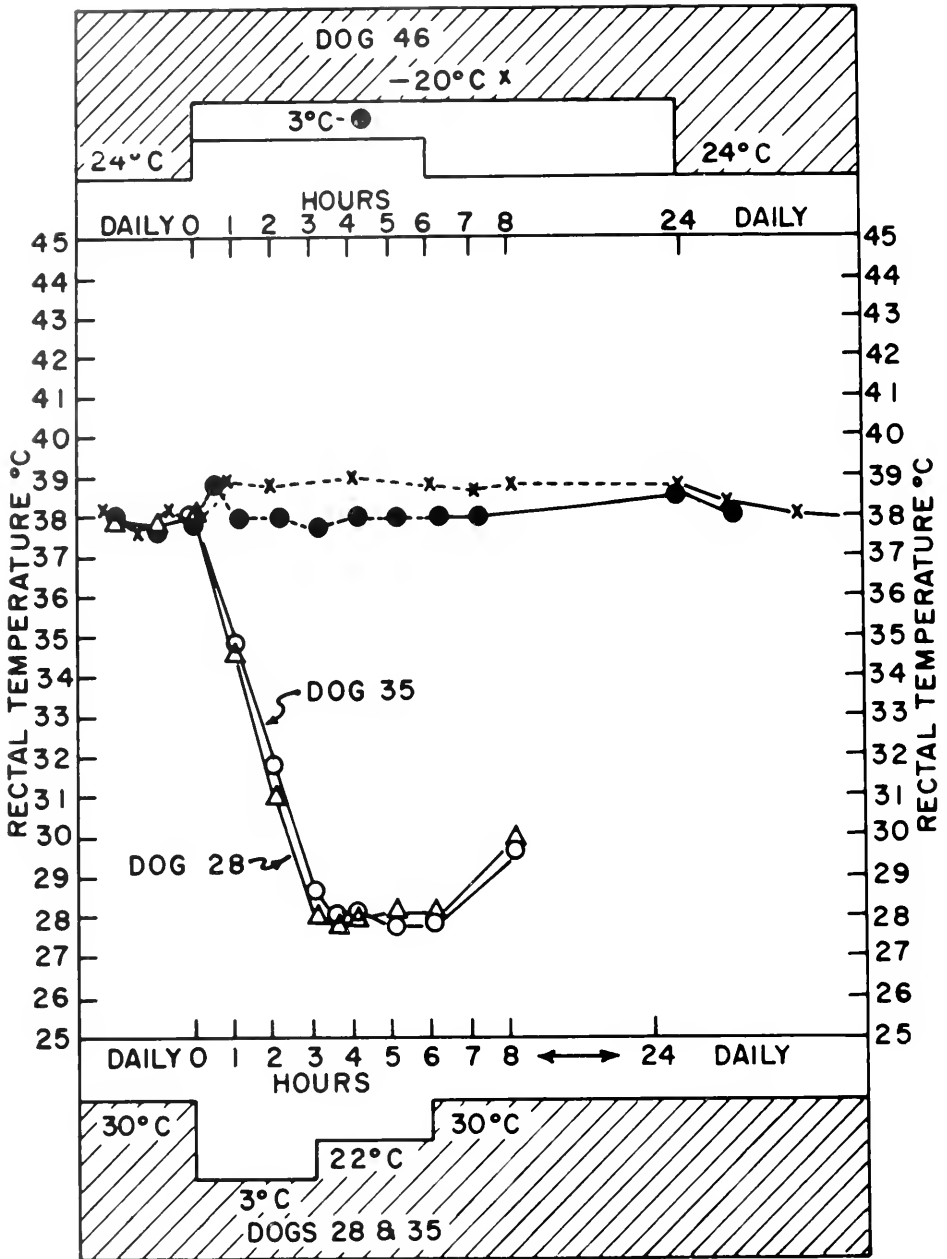


FIG. 1.—Upper Portion. Deep colonic temperature of a dog when it was subjected to (1) a moderate cooling load for 6 hours, solid circles, and (2) a heavy cooling load for 24 hours, crosses. The presence of shivering is indicated by stippling between temperature points. Note that the heavier the cooling load the more vigorous is the physiological resistance to core hypothermia. Lower Portion. Body cooling curves of two completely poikilothermic dogs when they were abruptly subjected to the same cooling load. Solid lines between temperature points indicate the absence of shivering. Note the straight line fall in body temperature while housed at 3° C, and absence of any spontaneous rise when removed to an ambient temperature of 22° C.

exposure; there was no shivering. This straight line fall in core temperature at the rate of 3.3°C . per hour with no change in slope that might indicate the retention of remnantal regulatory powers has been the criterion used for characterizing the completely poikilothermic animal. When the preparations were removed from the 3° to a 22°C . ambient temperature, core temperatures remained stationary at 28°C .

The tissue defect in each of these preparations was a reasonably selective thermo-coagulation of the posterior half of the hypothalamic gray. Sagittal sections taken from the series on Dog 28 are shown in figure 2 and illustrate the location and extent of the tissue defect which rendered this animal completely poikilothermic against cold. Observe the sharpness of the edges of the lesion with no distortion of adjacent tissue and the absence of debris or scar in the defect—it has simply become a part of the third ventricle. It has been our experience that a lesion which eliminates all regulation against cold does not leave any gray caudal or lateral to the lesion but in turn the defect need not involve the tissue lying immediately adjacent to this gray. If posterior hypothalamic gray remains, a proportional ability to combat cold also remains; it matters not whether the intact gray be situated laterally, medially, dorsally or ventrally. If a relatively large portion of the posterior hypothalamic gray remains undisturbed, the animal's regulation against cold does not deviate materially from the normal.

The partially poikilothermic animal. Cooling curves demonstrating retention of remnantal cold combatting powers are graphed in figure 3. A curve which has been interpreted as indicating the absence of non-shivering heat production with retained remnantal shivering heat producing ability is shown in the instance of Dog 112. For the first two hours the core temperature fell at the same rate as in the completely poikilothermic animal after which, at a core temperature of 31°C ., shivering began and sufficient heat was produced to slow body cooling materially. A curve suggesting the retention of remnantal non-shivering heat producing ability is shown in the instance of Dog 53. Here the core temperature fell progressively in essentially a straight line but more slowly than in the instance of a complete deficit; shivering was not in evidence at any time. A cooling curve which indicates the retention of both non-shivering and shivering heat producing ability in remnantal amounts is shown in the instance of Dog 527. At first the core temperature fell in a straight line but more slowly than in the instance of Dog 28, until it reached 32°C . at which time shivering began and rapidly produced sufficient heat to prevent further body cooling.

A slower cooling rate in the absence of shivering might be due to retained ability to reduce heat loss by conductance rather than to an increase in internal heat. However, the fact that such preparations exhibit a spontaneous rise in body temperature when removed to an ambient temperature of 22°C ., whereas the core temperature of the completely poikilothermic dog does not, has been interpreted by us as giving presumptive proof of retained non-shivering heat producing ability both in relation to hypothalamic ablations and localization of nerve fiber pathways at lower levels.^{3, 4}

Oxygen consumption determinations on such preparations have verified the correctness of this interpretation as illustrated below in figures 5, 6 and 7.

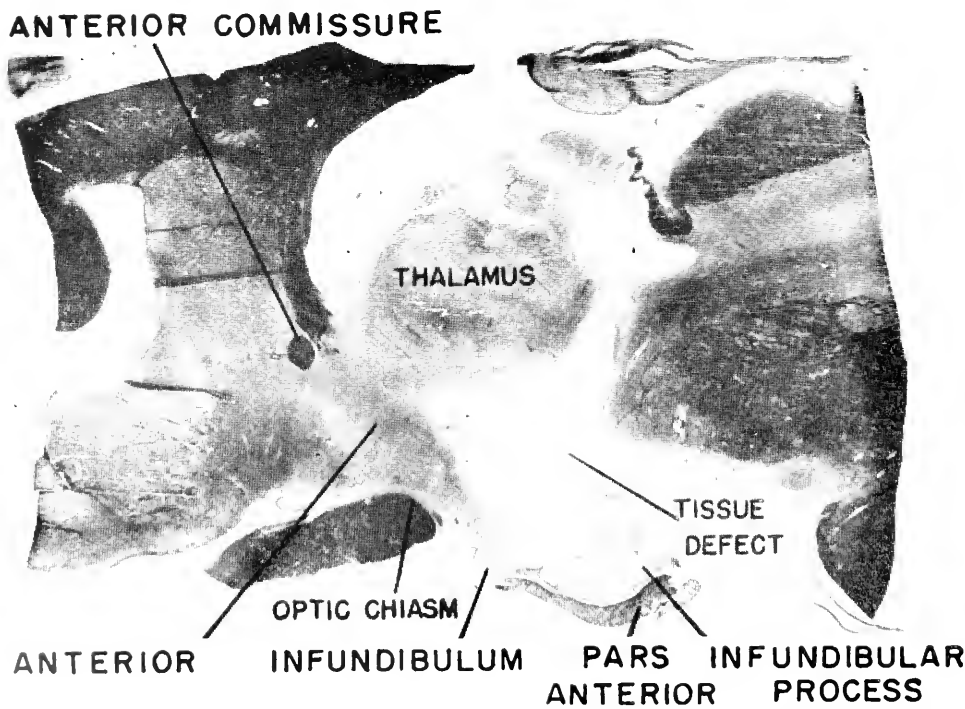
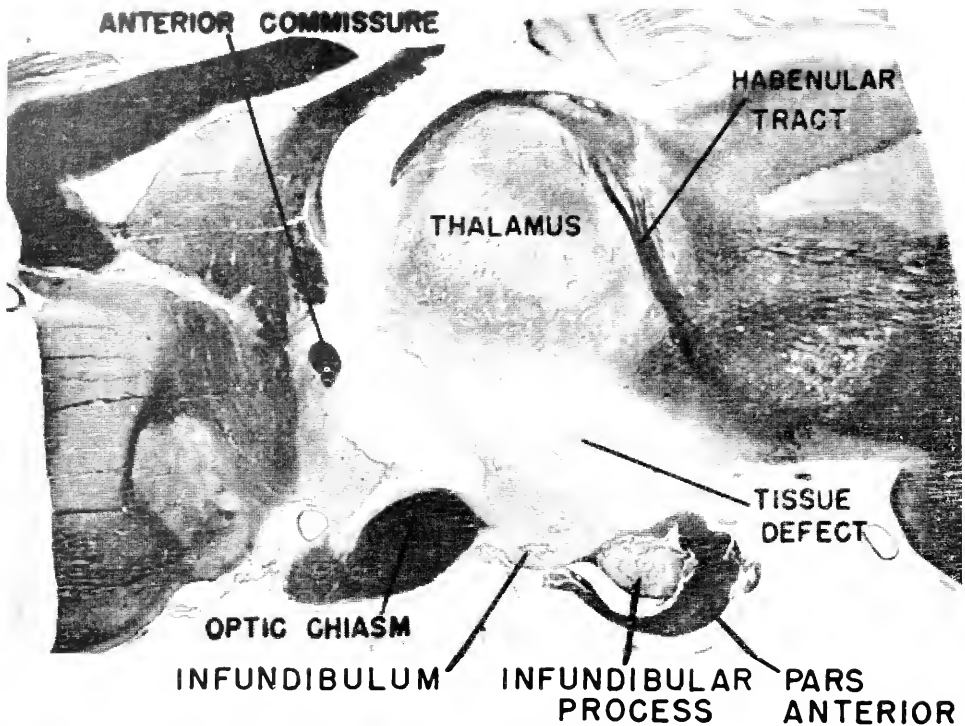


FIG. 2. Photographs of sagittal sections taken from the series on Dog 28 which show the location and extent of the tissue defect which eliminated all physiological resistance to hypothermia. The upper section is from near the mid-line, the lower section from a more lateral position. Note the absence of any distortion of the tissue located immediately adjacent to the tissue defect.

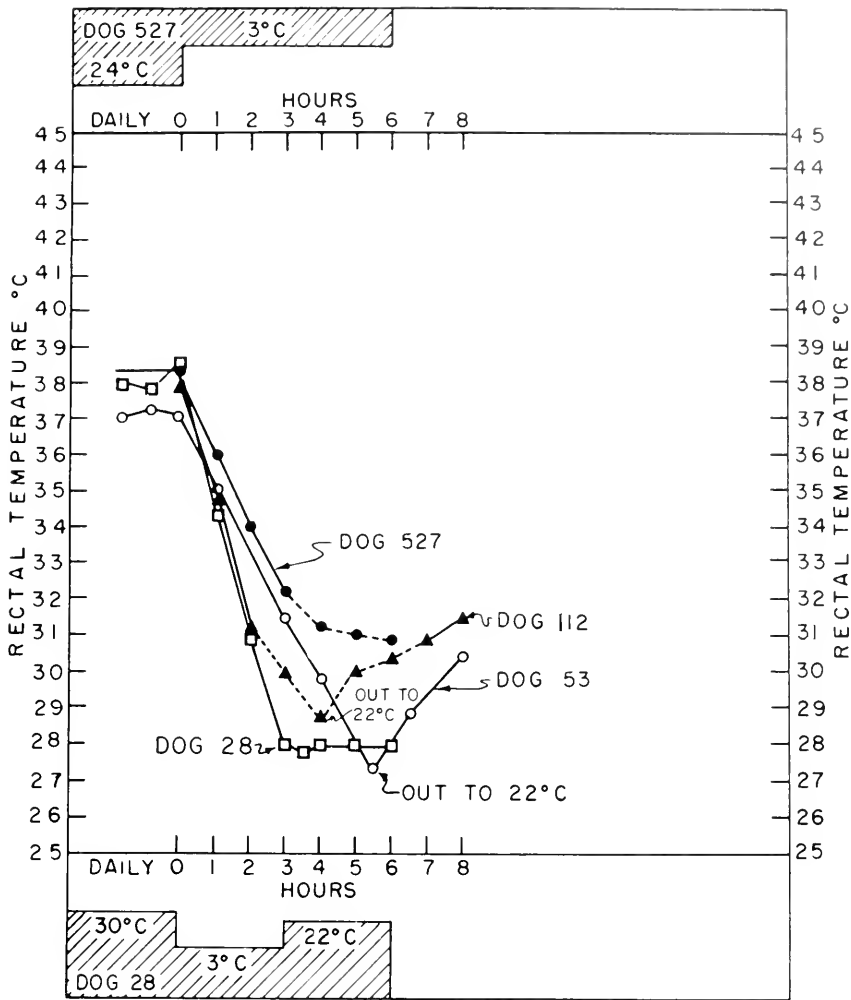


FIG. 3.—Cooling curves illustrating different types of remnantal cold combatting powers which frequently escape the surgical attempt to reduce the animal to the completely poikilothermic status. See text for further reference.

BASAL HEAT PRODUCTION* IN DOGS RENDERED POIKILO-THERMIC IN VARYING DEGREES BY LARGE POSTERIOR HYPOTHALAMIC COAGULATIONS

Basal heat production determinations calculated from oxygen consumption in 45 unoperated dogs, 36 dogs after they were inflicted with large posterior hypothalamic coagulations and 6 litter mates before and after varying degrees of adeno-hypophysectomy are plotted in figure 4. As shown by the lines drawn through the scattered points for the unoperated dogs the average basal heat production for the 5-13 kg. weight range was 1.75 Calories per kilogram of dog per hour, for the

* The author is indebted to Lt. Harold G. Danford for oxygen consumption determinations.

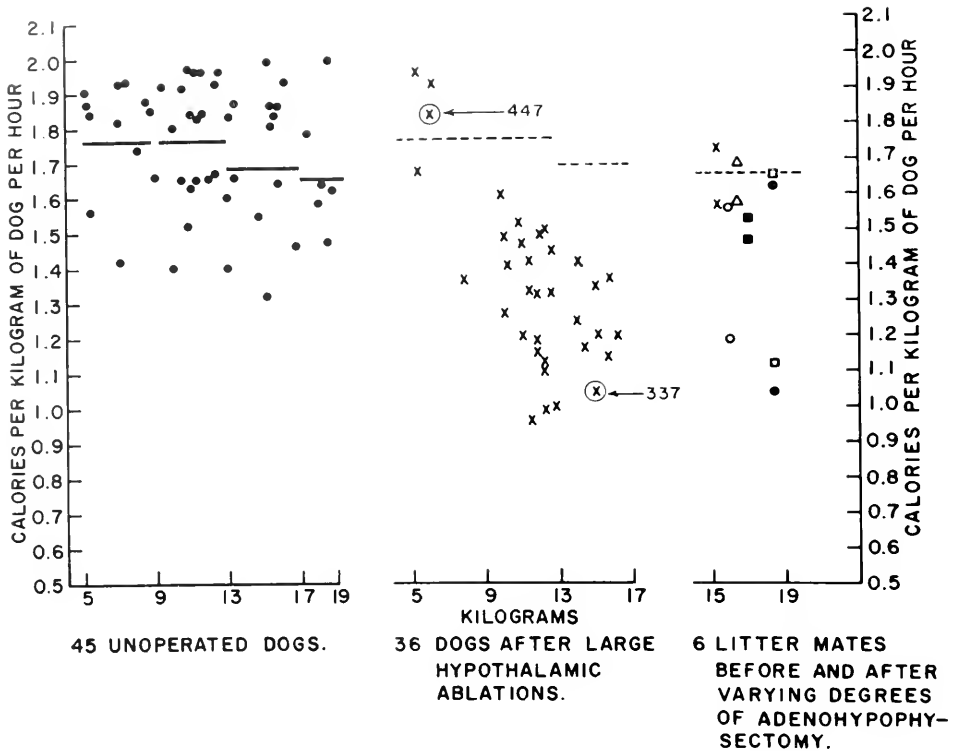


FIG. 4.—Basal heat production values as calculated from measured oxygen consumption. To the left, designated by solid circles, are the individual values for 45 unoperated dogs. In the center are individual values for 36 animals after large hypothalamic ablations, designated by crosses. To the right are values for 6 litter mates before and after varying degrees of adeno-hypophysectomy; a pituitary stalk section designated by crosses, partial hypophysectomies designated by open triangles and solid squares and near-total hypophysectomies by solid circles, open squares and open circles.

13–17 kg. weight range 1.70 Calories and for the 17–19 kg. weight range 1.65 Calories.

The hypothalamic coagulations reduced basal heat production materially in all but 5 dogs (5–8) and in four instances to the level of 1 Calorie per kg. per hour which is in the neighborhood of a 40 per cent reduction and is equal to the maximal lowering which follows an appropriate adeno-hypophysectomy. Encountering a graded reduction in basal heat production in a group of dogs which also exhibit a graded impairment in ability to regulate against cold at first sight might cause one to suspect that these deficits are directly related; particularly because of the assumption so widely expressed in the literature that endogenous epinephrine and thyroxin are utilized to increase non-shivering heat production during cold exposure. Quite to the contrary, the two deficits seem not to be related, because dogs can be rendered completely poikilothermic against cold without any associated decrease in basal energy metabolism. Also, an animal which suffers a maximal reduction in basal heat may retain considerable cold-combatting powers. Dog 447 circled in the chart

in figure 4 is an example of the former group and 337 also circled is an example of the latter group.

The differential lesions are schematized in the drawing in figure 5. When the junctional tissue lying between the hypothalamus and midbrain is coagulated, as delimited by the stippled circle, without infringing materially upon the structural integrity of the hypothalamic gray the animal is rendered poikilothermic without any lowering in basal energy metabolism. This same result is attained by complete transection of the brain stem at any level below the hypothalamic gray as indicated by the straight line. But, when the heat-regulating nerve cells are ablated at the expense of considerable cephalic encroachment upon the structural integrity of the tuberal and anterior hypothalamic gray as delimited by the solid circle, a reduction in basal energy metabolism results in addition to rendering the animal poikilothermic.

Analysis of these differential tissue defects and associated deficits forces the conclusions that physiological regulation against cold is primarily a neural affair, a function of the *neural hypothalamus*, whereas the normally elevated basal energy metabolism is an endocrine affair, presumably a function of the *endocrine hypothalamus*.

The possibility that the basal energy metabolism deficit might be directly due to "neighborhood" involvement of the adenohypophysis cannot be entirely ruled out. However, it is doubtful that this is the case because these preparations exhibit no other hypophysial deficits (except possibly sex atrophy) and further it is necessary to remove the larger part of the adenohypophysis before a basal energy metabolism

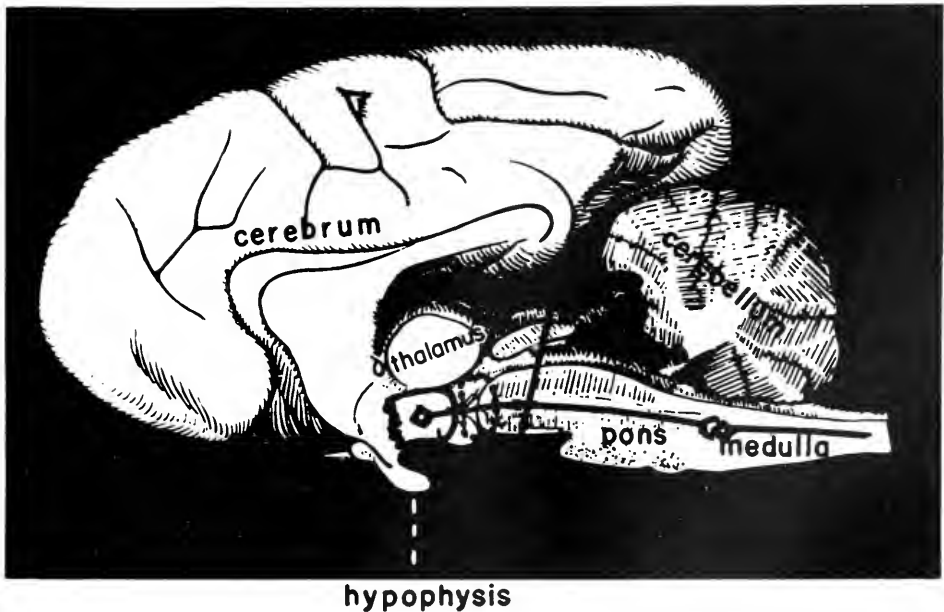


FIG. 5.—Diagram schematizing the differential lesions for separation of mechanism for regulation against cold and the mechanism for reduction in basal energy metabolism. See text for further reference.

deficit is in evidence (see data in figure 4). It is not a matter of the adenohypophysis being dependent upon nerve impulses passing from the hypothalamus to the hypophysis through the infundibulum because the adenohypophysis can be completely separated from the hypothalamus either by simple stalk section (Dog 295, figure 3) or by a selective neurohypophysectomy⁹ without altering basal energy metabolism.

It seems reasonably certain that this reduction in basal energy metabolism precipitated by associated massive involvement of the hypothalamic gray matter is the underlying basis for (1) an increased sensitivity to anesthesia exhibited by these preparations, (2) the decrease in the magnitude of a neurohypophysectomy polydipsia,¹⁰ (3) the enhancement of pathological obesity when animals are maintained on a fixed diet¹¹ and perhaps the permanent lowering of blood pressure both in the normotensive and renal hypertensive dog,¹² as well as the increased threshold for eosinopenic stimuli associated with hypothalamic lesions of various categories.¹³

Temperature coefficient for heat production in poikilothermic dog. The temperature coefficient for heat production in the completely poikilothermic dog cannot as yet be stated with exactness, first, because of the small number of animals studied and, second, because of the complication which a reduction in basal energy metabolism introduces into the calculations. Heat production values at different body temperatures are plotted in figure 6 opposite the cooling curves for 4 animals, 2 of which were completely poikilothermic against cold and 2 which retained remnantal non-shivering heat producing ability.

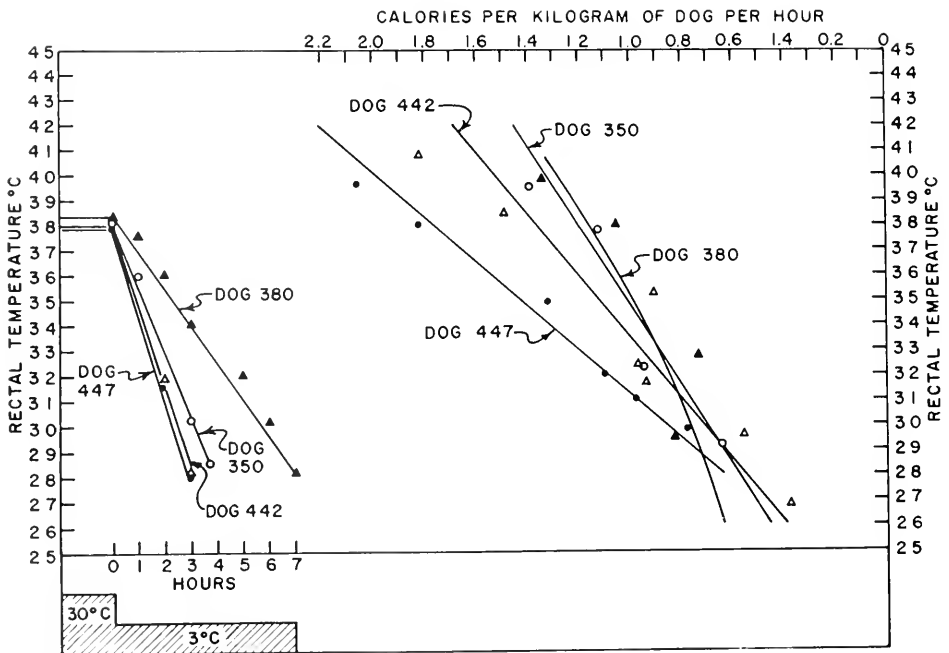


FIG. 6.—Cooling curves on left and heat production values for various body temperatures on right for four dogs. Note that two were completely poikilothermic and two retained remnantal non-shivering heat producing ability, as demonstrated by the cooling curves as well as the heat production data. See text for further reference.

Dog 447 and Dog 442 were both completely poikilothermic according to the criterion of cooling to 28° C. in three hours. Note that the lines fitted through the heat production points for the 2 animals both show a progressive straight line fall but that the slope of the curve for 447 is steeper than the one for 442; 447 having a calculated Q_{10} of 2.8 and 442 one of 2.5. This difference in Q_{10} seemingly is due to the fact that 447's basal heat production was in the normal range, whereas 442 suffered a 20 per cent reduction and at lower body temperatures *the spread between the heat production values progressively decreased*. (It also follows that the elevated basal energy metabolism mediated by the endocrine hypothalamus is maximally effective only at the homotherm temperature level.) Thus, one can roughly predict that the Q_{10} for a completely poikilothermic dog with an associated maximal reduction in basal heat will be in the neighborhood of 2.2. Accordingly, in using the Q_{10} as a criterion for evidencing the absence of or the retention of a non-shivering cold stimulated internal heat source it is necessary to take into account the presence and magnitude of any associated reduction in basal energy metabolism.

Cold stimulated non-shivering heat production. The cooling curve on the other 2 dogs in figure 6 reflects the retention of remnant non-shivering cold stimulated heat production. In Dog 350 it took an extra hour and in Dog 380 an extra four hours to cool to 28° C. The calculated Q_{10} was 2.1 for 350 and 1.6 for 380. Both figures correlate well with the degree of retained heat-producing ability indicated by the cooling curves.

Other examples of close correlations between cooling curves and calculated heat production values at different body temperatures are illustrated in figures 7 and 8 by Dogs 396 and 337. In Dog 396 the body cooled during the first hour at the rate of a completely poikilothermic animal after which the curve moved decidedly to the right. Similarly, heat production at a core temperature of 35° C. indicated a fall in heat production at the rate of a Q_{10} in the same range of that for the non-heat regulating animal but subsequently plateaued such that there was as much heat being produced at a core temperature of 30° C. as there was at 33° C. This demonstrates the presence of a sizeable remnant of non-shivering heat producing ability which was activated at a considerably increased threshold. Further, the amount of heat produced was progressively increased as body temperature was lowered (see also heat production points for Dog 380 in figure 6). In spite of a maximal reduction in basal oxygen consumption Dog 337 exhibited both non-shivering and shivering heat producing ability, exhibiting a Q_{10} in the neighborhood of 1.6 for heat production up to the time when shivering was activated.

The subject of cold-stimulated non-shivering heat production requires further comment. There has long been a controversy as to whether there is an automatic increase in non-shivering heat production in response to cold. Analysis of the foregoing data should leave no doubt as to the existence of a non-shivering heat source in the dog. *It should be emphasized that cold-stimulated, non-shivering heat production is just as distinct an entity as is shivering heat production.* Each has separate and distinct nerve cell and descending fiber tract representation in the hypothalamic gray matter and brain stem. Each is equally dependent upon nerve impulses efferenting from these neurons. Following hypothalamic ablations remnants of each may be disassociated one from the other and may exhibit a markedly increased

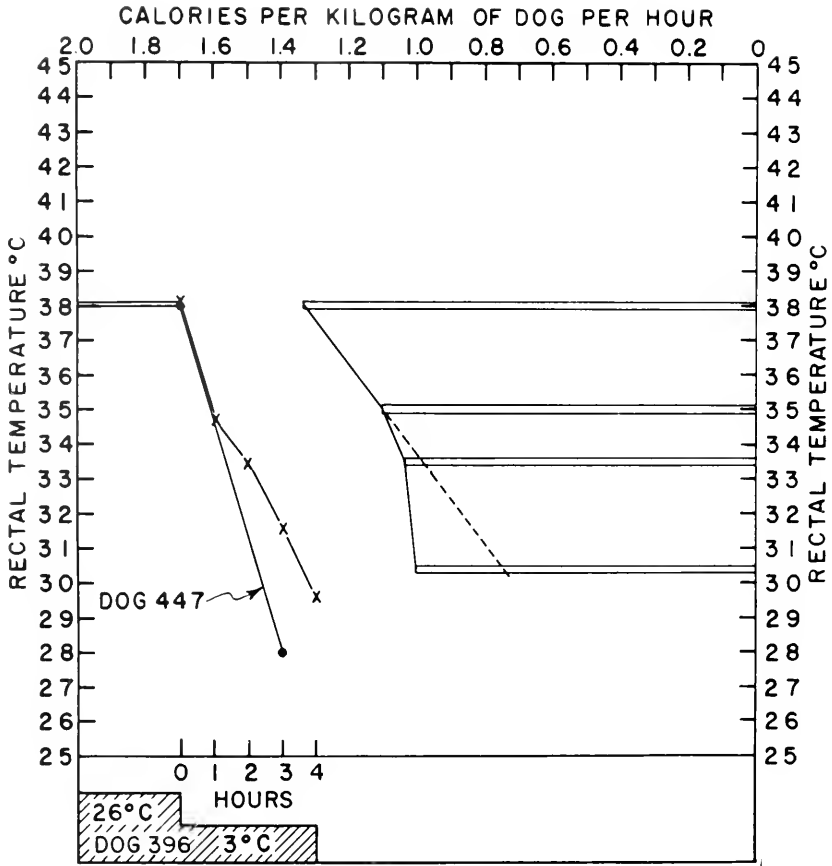


FIG. 7.—Cooling curve and heat production values for different body temperatures, graphed as bars, for Dog 396 evidencing (a) retention of non-shivering heat producing ability and (b) a definitely raised threshold (lower body temperature) for activating the heat elaborating process.

(lower body temperature) activation threshold. Each is superimposed upon and entirely independent of basal energy metabolism. The site of elaboration of shivering heat is known to be in striated muscle but it is important to remember that muscular contraction of a specialized type is specifically utilized for this purpose. The site of the elaboration of non-shivering heat production is not known but this information as well as an insight into the mechanism of its elaboration should be forthcoming from preparations of the type described.

OBSERVATIONS ON THE EFFECT OF HYPOTHERMIA ON MISCELLANEOUS ORGANS

The program for determining the effects of thermal variables on individual organs and/or systems in the unanesthetized poikilothermic dog has not progressed beyond an orientation stage. Accordingly, statements made below are entirely preliminary in scope.

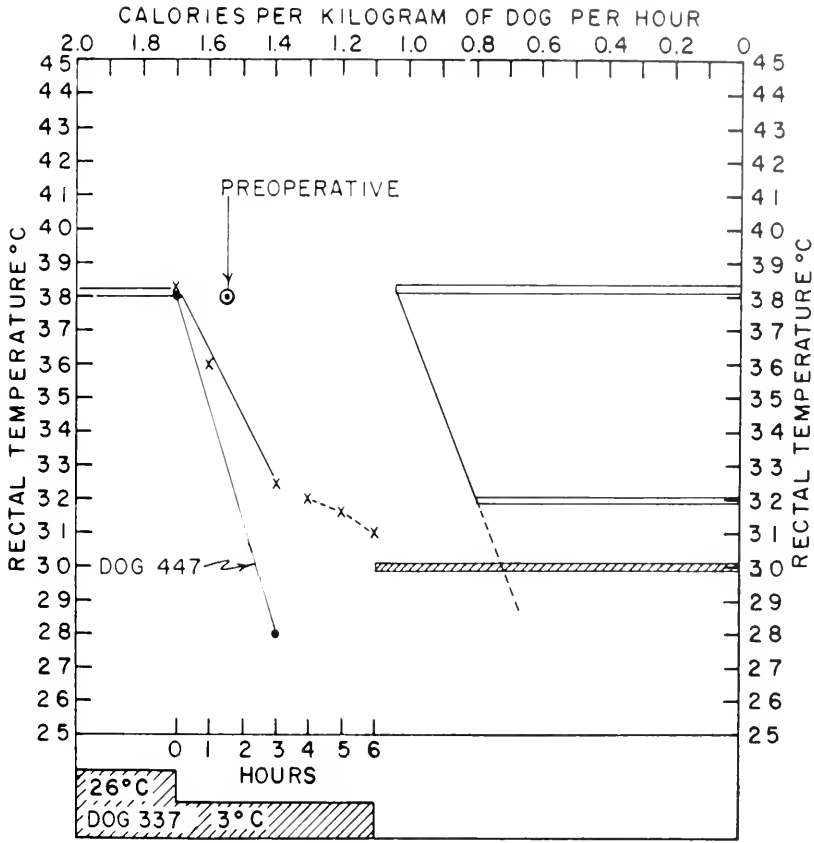


Fig. 8.—Cooling curves and heat production values for different body temperatures, graphed as bars, for Dog 337, evidencing retention of both non-shivering and shivering heat production in remnant amounts. The presence of shivering is indicated by stippling in the cooling curve and by cross hatching in the heat production bar.

The circled point is the preoperative basal heat production value for this particular dog. See text for further reference.

Renal.[†] All procedures were carried out in the post-absorptive state. Hypothermia was induced by subjecting the animal to an ambient temperature of 3° C. until deep colonic temperature was reduced to the desired level, after which the animal was moved to a room held at 20 to 22° C. Body temperature remained at a reasonably constant level during the time that renal clearances were determined. Clearances at different body temperatures were done on different days with no less than 5-day intervals. Laboratory procedures have been described in detail elsewhere.⁹

Glomerular filtration rate and effective renal plasma flow were determined in 5 dogs and Tm PAH in 4 dogs, all of which retained varying degrees of remnant physiological resistance to hypothermia but not sufficient in magnitude to prevent a progressive hypothermia from developing when exposure to cold was sufficiently prolonged.

[†] The author is indebted to Lt. Ivan J. Mader for renal function assessments.

There was an obvious decrease in glomerular filtration rate and renal plasma flow which progressed more rapidly at lower body temperatures (32 to $28^{\circ}\text{C}.$) than at the mild hypothermia level (36 to $34^{\circ}\text{C}.$). This tendency for the clearances not to decrease at lesser degrees of hypothermia seemed to be roughly proportional to the magnitude of the retained cold-combatting powers. The results obtained on a dog which approximated closely the completely poikilothermic status and which also did not exhibit any postoperative change in basal energy metabolism are charted in figures 9 and 10. The results in this dog perhaps reflect the effects of graded hypothermia upon these functions, uncomplicated by other superimposed factors. In both instances the clearances were slightly higher postoperatively but they decreased progressively, in a reasonably straight line fashion, with a progressive decrease in body temperature.

Tubular maxima for para-aminohippurate were significantly lowered at reduced

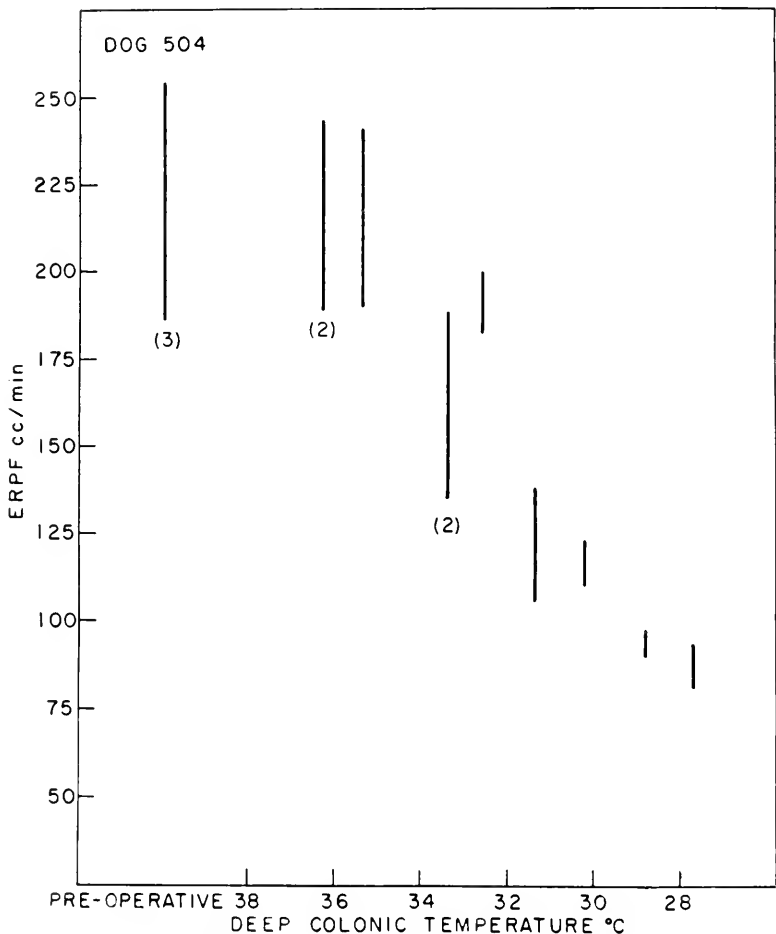


FIG. 9.—Relationship of effective renal plasma flow to deep colonic temperature in Dog 504, a preparation which approached the complete poikilotherm. Figures in parentheses indicate number of times clearances were done at that particular core temperature.

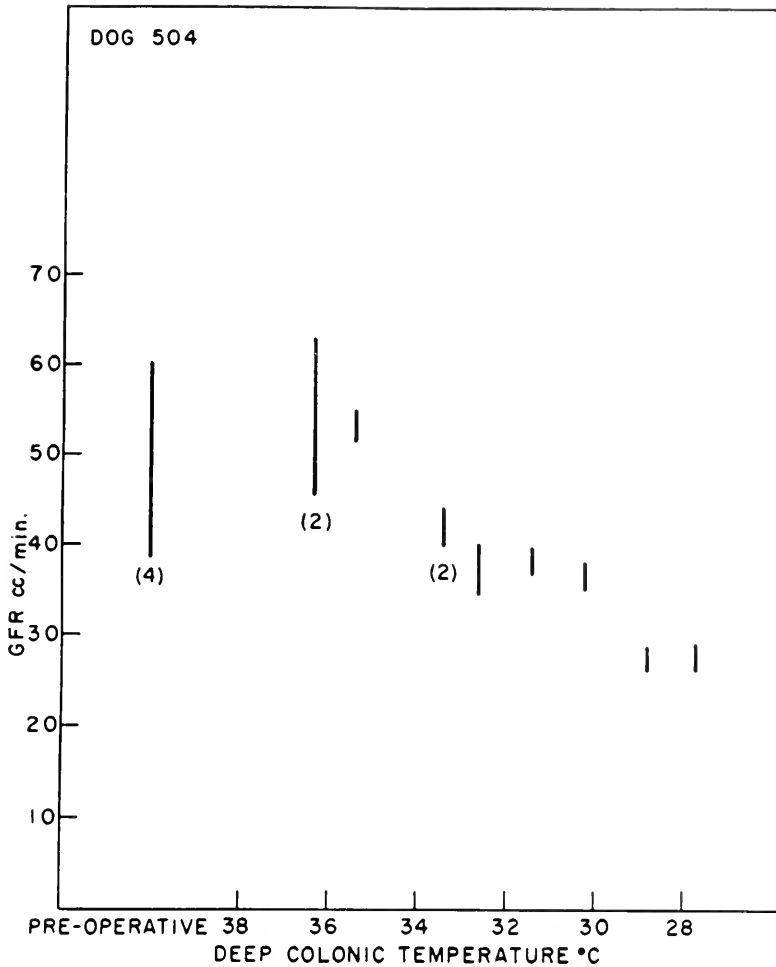


FIG. 10.—Relationship of glomerular filtration rate to deep colonic temperature in Dog 504. Figures in parentheses indicate number of times clearances were done at that particular core temperature.

body core temperatures, but in contrast to glomerular filtration rate and renal plasma flow in most instances, the greatest reduction occurred characteristically at mild degrees of hypothermia. The data on 4 dogs are charted in figure 11. Here again the decrease in tubular maxima in the dog, which approximated a straight-line fall, was in a preparation which approximated the completely poikilothermic status. Perhaps, therefore, the precipitous fall during early hypothermia can be correlated with the retention of remnantal heat regulatory ability.

The ability of the kidney to concentrate, as measured by osmotic flow-load curves, was not noticeably affected by core temperatures of 28° C.

Cardiovascular.[‡] Observations were made before and during the induction of hypothermia and during rewarming in several unanesthetized poikilothermic dogs

[‡] The author is indebted to Lt. Frederick R. Mugler, Jr. for cardiovascular observations.

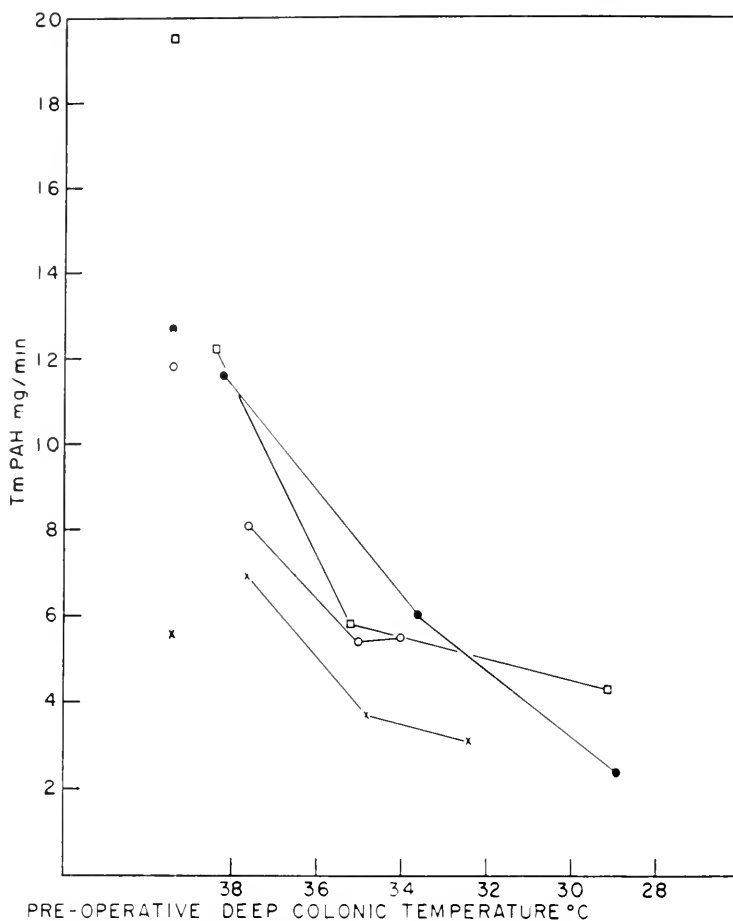


FIG. 11.—Relationship of tubular maxima for para-aminohippurate to deep colonic temperature in four partially poikilothermic dogs. The variation in slope may be related to retained resistance to hypothermia; note that the line drawn through the solid circles approaches a straight line and this animal retained the least cold combatting powers.

and several unoperated animals when under a surgical depth of nembutal anesthesia. Hypothermia was induced by subjecting the animals to an ambient temperature of 15 to 10° C.; rewarming was accomplished by rapidly increasing the temperature of the room to 40 to 45° C. All the unanesthetized dogs retained some ability to resist hypothermia but not sufficient in magnitude to prevent a progressive hypothermia from developing when the cold exposure was sufficiently prolonged.

The electrocardiograms have shown the expected P-R and Q-T prolongation, with a broadened QRS, on cooling the poikilotherms. Additionally, the ST segment current of injury has been noted when the deep colonic temperature dropped below 33° C.

The poikilothermic dogs have uniformly exhibited a postoperative bradycardia and sinus arrhythmia with marked fluctuation in systolic and diastolic blood pressure levels, depending on the respiratory phase. On cooling, a gradual reduction of the

mean blood pressure has occurred, with further slowing of the bradycrotic pulse. On rewarming, the mean blood pressure rose but the pulse rate did not rise and at times continued to decrease. With the onset of shivering, a rise in blood pressure has consistently been observed. Also on cooling, respiration has slowed in all animals and on rewarming has returned to or above the initial rate. These features are illustrated by the data from one experiment charted in figure 12.

In the poikilotherms, the temperature of the paw toe pads has fallen sharply to near that of the cool ambient air. The chest skin temperature has varied from 2 to 8° C. below the deep colonic temperature. On rewarming, these skin temperatures have *risen* sharply toward the ambient air temperature of 40–45° C. These features are illustrated by the data from one experiment charted in figure 13.

During cooling the operated dogs consistently showed a rise in arterial whole blood CO₂ while the changes in arterial whole blood pH, hematocrit and arterial oxygen saturation have been variable. These features are illustrated by the data from one experiment charted in figure 14. In our experience the anesthetized unoperated dog with unassisted respiration has shown an earlier and greater alteration in the chemical status of the blood during cooling than have the poikilotherms; in particular, the pH fall has been more constant and CO₂ rise more marked.

Some of the poikilothermic animals have shown irritability and excitement at the

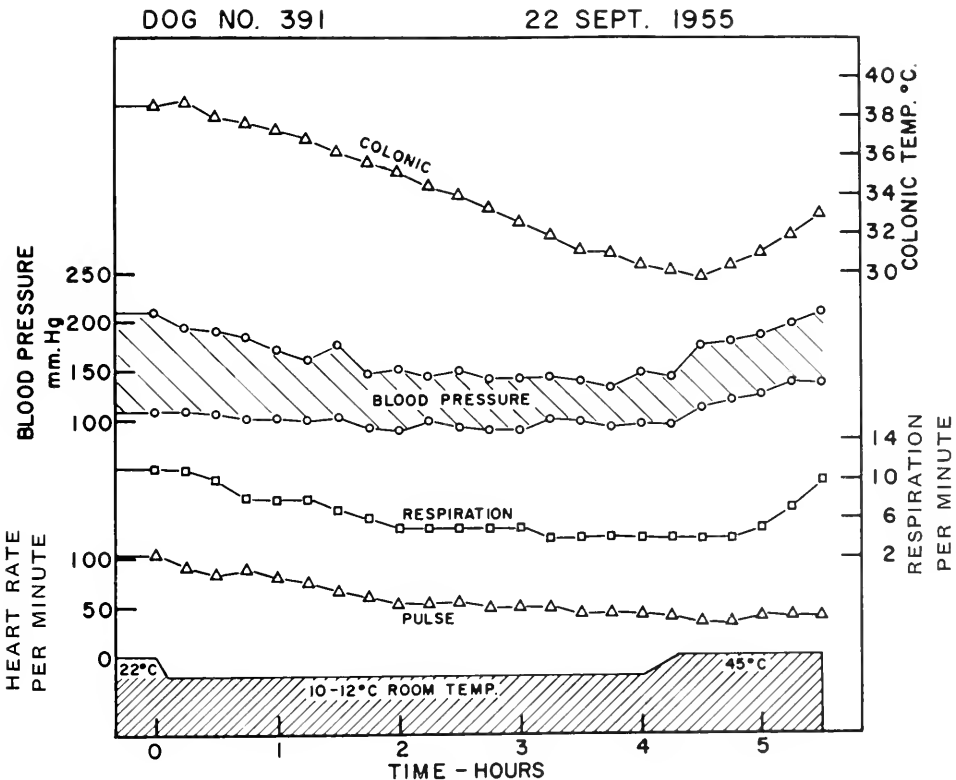


FIG. 12.—Diagram of vital signs on cooling in a representative experiment.

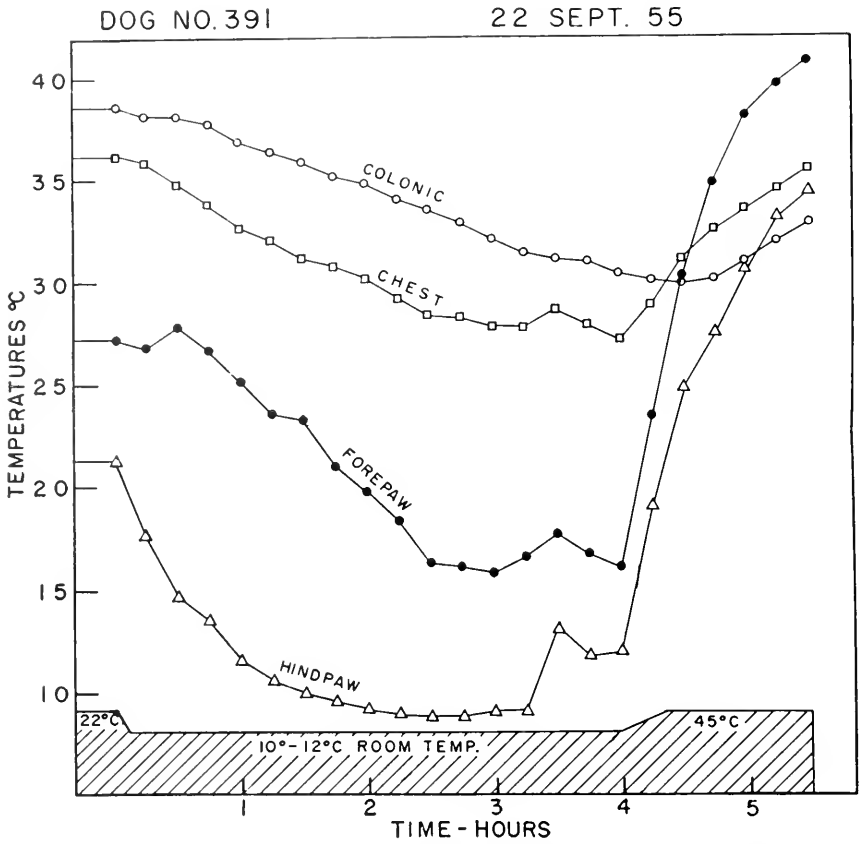


FIG. 13.—Skin and deep colonic temperatures on cooling in a representative experiment.

hypothermic range of 30 to 32° C. One dog displayed such irritability at a core temperature of 34° C. induced by minimal change in the position of the colonic thermocouple, consistently causing an abrupt systolic blood pressure elevation of 50 to 60 mm. Hg. and a diastolic elevation of about 40 mm. Hg.

Four dogs died while routine cooling curves were being run. Their deaths were characterized by sudden collapse. Artificial respiration and massage of the chest were of no avail. One dog with a colonic temperature of 29° C. died after a bout of exercise, jumping rapidly from the cage to the floor of the room, whereas, in a previous cooling run it had survived a core temperature of 27° C. Shivering was present in the other 3 dogs at the time of their death, all of which occurred when the core temperature was 28° C. It seems reasonably certain that ventricular fibrillation was the cause of death in these animals.

Mental awareness, movements of progression and related functions. Central nervous and neuromuscular functions are not seriously disturbed by body temperatures as low as 28° C. This is reflected by the fact that the animal remains fully oriented with its surroundings; it may jump eagerly from the cage to the floor, smell out food, jog to food thrown across the room, chew and swallow without any obvious disturbance in deglutition or digestion, refuse feces as food and respond

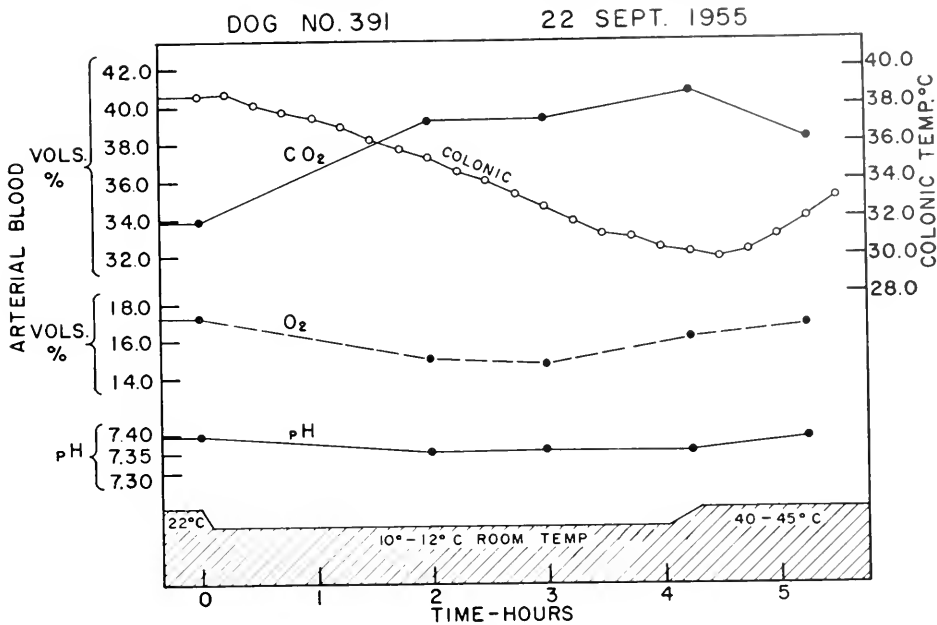


FIG. 14.—Serial whole blood pH, CO₂ and arterial O₂ on cooling in a representative experiment.

emotionally to being teased or stroked. The animals exhibit no outward signs of discomfort from cold.

Disturbance in movements of progression are not discernible until body core temperature reaches the neighborhood of 34° C., at which point there is a suspicion of a lack of smoothness in muscular movement. At 32° C. there is a definite slowness in muscular movement with a beginning appearance of a characteristic "gait." At 30° C., superimposed upon the "gait" which is now definite, is an occasional stagger to one side or the other with automatic "catching" of the body to prevent falling. At 28° C. the immediately foregoing irregularities may or may not be noticeably intensified. It is suspected that this variation at 28° C. in the severity of symptoms from one animal to another is a function of the *duration* of core hypothermia.

In general it is surprising that an animal is so little disturbed by a body temperature of 30 to 28° C. Analysis of the foregoing forces the conclusion that a 10° C. reduction of body temperature below the homothermic level does not eliminate or materially impair functions associated with cerebration, central and peripheral synaptic conduction and the contractile process in muscle. The staggering and "catching" of the body is interpreted as evidence of a beginning disturbance in the postural reflexes, presumably at the central synapse.

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DISCUSSION

Dr. Alan C. Burton: I would like to ask Dr. Keller if he has any observations on the effect of these surgical procedures on the pilo-motor response to cold.

Dr. Keller: The first dog shown in the movie exhibited pilo-erection at the same time that it began shivering; this was at a colonic temperature in the neighborhood of 32° C. The second dog shown exhibited no pilo-erection and did not shiver. Whether or not pilo-erection can be disassociated from shivering as a neurological remnant I do not know. It is difficult to tell on some animals whether pilo-erection is or is not present.

Dr. Burton: I was wondering if you felt that it was mediated by adrenal or by nerve supply.

Dr. Keller: Definitely by nerve impulses efferent from the hypothalamic level.

Dr. John W. Severinghaus: Did you measure the temperature of the dog in other parts of the body?

Dr. Keller: Yes, but we have not done so sufficiently to give a differential tabulation.

Dr. John W. Severinghaus: I asked about temperature gradients in various portions of the body because of a curious observation we have made. During cooling, the rectal and colonic temperature of a dog falls slightly faster than the arterial and esophageal temperature. However, after vagotomy, the temperature in a certain area of the colon, about 15–20 cm. from the anus, uniformly falls much faster than any of the other deep temperatures. We have observed temperatures 12–14 degrees cooler than the arterial, esophageal, and 5 cm. rectal temperatures. This result could be obtained by the use of ice water on the abdominal wall alone. However, it was not due to direct heat transfer through the thin layer of wall, since the thermocouple at autopsy was far from the surface. There was no measurable fall in portal flow with vagotomy, so we do not believe this fall to be due to lack of blood flow to the colon. Our unproven hypothesis is that after vagotomy, blood from the abdominal wall may return to the systemic circulation by way of veins closely associated with this area of the colon. It may be, therefore, that other neurologic lesions might produce this anomaly and give erroneous temperature recordings if deep rectal temperature alone is measured.

Dr. Jean Cahn: Did you cool thyroidectomized animals?

Dr. Keller: No, we have not.

Dr. Cahn: We did many experiments on adrenalectomized or hypophysectomized rats, either in surface cooling technique or in artificial hibernation, and we observed the same results. But we found that it is very difficult to cool some thyroidectomized animals. They resist the cooling even more when the thyroidectomy has been done for two or three days.

It would seem the resistance against cold is greatest after three or four or five days, and it is impossible to obtain the same drop in the body temperature as in the control if we don't block the thyrotropic secretion by iodine proteins (tyrosine or thyroxine). If we did, we obtained a drop in the colonic temperature for thyroidectomized animals similar to that obtained in the control.

PHYSIOLOGY OF HIBERNATION IN MAMMALS

CHARLES P. LYMAN* AND PAUL O. CHATFIELD

In reviewing the various physiological problems of hibernation in mammals, one is hampered by the fact that the word "hibernation" is not sufficiently precise in meaning. Webster defines "to hibernate" as "to pass the winter in close quarters in a torpid or lethargic state." Such a definition must include all groups of animals which are somewhat less active in the winter than they are in the summer. The present article concerns itself with a small number of mammalian species which at some time of year undergo a profound drop in body temperature with a concurrent decrease in metabolism and heart rate. Because there is no single word which describes this condition, it is perhaps best to refer to it as "deep hibernation," but for the sake of brevity the word "hibernation" will be used in this restricted sense.

Good comparative physiological data on mammals that hibernate are scanty, and it appears likely that many animals which are commonly included in this group cannot be considered as "deep hibernators." For example, the brown bear, *Euarctos americanus*, spends part of the winter in a dormant state but shows no striking drop in body temperature or metabolic rate.^{1, 2†} Clapp's³ observation of vapor rising from the nostrils of a dormant bear during the winter also indicates that the body temperature is much higher than that of the environment. It appears probable that there are gradations of dormancy from the heavy sleep of the bear to the deep hibernation typical of some smaller mammals, but only the latter category will be considered here. Table I gives a list of the more available animals which are known to be deep hibernators. Throughout the remainder of this article animals will be referred to by their "common" English names.

It will be noted that several species of bats have been included in this table. As Hock⁹ has pointed out, this order of mammals stands alone among the hibernators, for their body temperature and metabolic rate drop precipitously whenever they become inactive. Apparently the hummingbird¹⁰ and the poor-will¹¹ belong in the same category as bats. On the other hand, the rodents and insectivores which hibernate can be exposed for long periods to a cold environment without changing their body temperature. After hours, or sometimes months, the individual animal abandons its homeothermic state, the metabolic rate decreases and body temperature drops, and hibernation begins. Because the factors involved in these two types of hibernation may not be the same, this review will be limited for the most part to hibernation in rodents and insectivores.

It should be emphasized here that there has always been some confusion between hibernation and hypothermia. This confusion has grown with the advent of experimental hypothermia for surgical purposes and the development of pharmacological products which will permit hypothermia.¹² The differences between the two states have been clarified at length by Popovic¹³ and Giaja.¹⁴ Any mammal, if exposed to a sufficiently low temperature, will increase its muscular activity, shiver and increase

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† See also Svihla, A. and Bowman, H. C.: *Am. Mid. Nat.* 52: 248, 1954.

TABLE I

Common name	Scientific name	Synonym	Insectivores			Partial list of investigators
			French	German		
Hedgehog	<i>Erinaceus europaeus</i>		Herisson	Igel	Adler ¹⁰ Suomalainen ¹⁰	
Rodents						
Marmot	<i>Marmota marmota</i>	<i>Arctomys marmota</i>	Marmotte	Murmeltier	Dubois ¹² Kayser ¹⁸	
Woodchuck	<i>Marmota monax</i>	<i>Arctomys monax</i>	Marmotte	Waldmurmeltier	Rasmussen ¹⁵ Benedict and Lee ¹³ Kayser ¹⁸	
European hamster	<i>Cricetus cricetus</i>	<i>Cricetus frumentarius</i>	Hamster des champs	Hamster	Kayser ¹⁸	
Golden hamster	<i>Mesocricetus auratus</i>	<i>Cricetus auratus</i>	Hamster doré	Goldhamster	Kayser ¹⁸ Chatfield and Lyman ¹⁰	
Common dormouse	<i>Glis glis</i>	<i>Myoxus glis</i>	Loir	Sieben schläfer	Kayser ¹⁸ Wys ⁸⁴ Kayser ¹⁸	
Garden dormouse	<i>Eliomys quercinus</i>	" <i>Myoxus arbor</i> "?	Lérot	Gartenschläfer	Kayser ¹⁸	
Hazel "mouse"	<i>Muscardinus avellanarius</i>	<i>Myoxus avellanarius</i>	Muscardin	Haselmaus	Kayser ¹⁸	
European ground squirrel	<i>Citellus citellus</i>	<i>Spermophilus citellus</i>	Spermophile	Ziesel	Kayser ¹⁸	
Thirteen-lined ground squirrel	<i>Citellus tridecemlineatus</i>	<i>Spermophilus tridecemlineatus</i>	Ecreuil terrestre		Johnson ⁶¹ Foster ¹⁷ Zalesky ³⁷	
Arctic ground squirrel	<i>Citellus parryi</i>	<i>Spermophilus (Colobotis) parryi</i>	Ecreuil terrestre	Parrys ziesel	Svihla ⁶¹ Musacchia and Wilber ⁸¹	
Bats						
Scrotime bat	<i>Eptesicus serotinus</i>	<i>Vespertilio serotinus</i>	Scrotime		Courcier ¹¹⁴	
Long-eared bat	<i>Plecotus auritus</i>	<i>Vespertilio auritus</i>	Oreillard	Ohrenfleidermaus	Kayser ¹⁸	
Noctule bat	<i>Nyctalus noctula</i>	<i>Vesperugo noctula</i>	Noctule	Abendsegler	Merzbacher ¹¹⁸	
Little brown bat	<i>Myotis lucifugus</i>	<i>Vespertilio lucifugus</i>	Marin	Mausohren	Hock ⁶	

English names of European rodents from Simpson.⁴
 Scientific names of rodents from Ellerman.⁵
 English and scientific names of bats from Allen.⁶
 Common German names from Brehm⁷ and French names from Bonnière.⁸

its metabolic rate. If the cold is too intense or of too long duration, exhaustion eventually takes place and the animal begins to cool. When a critical level of body temperature is reached, death occurs. This sequence of events can occur in both hibernators and mammals that do not hibernate, but death takes place at a much lower body temperature in the former.¹⁵ Similar conditions can be realized by anaesthetizing an animal and exposing it to cold.

One important factor which characterizes the deeply hibernating state is that the normal animal is capable of arousing from this state without the aid of heat from external sources. Any hibernating mammal, having received a stimulus strong enough to start the process of arousal, will begin a chain of physiological reactions which result in a rapid production of heat and culminate with the animal fully awake at the end of two or more hours. Failure to realize that this is typical of true deep hibernation has led to some confusion in the past. For example, hibernators treated with drugs such as pentobarbital sodium and then exposed to cold cannot be considered to be in the deeply hibernating state when their body temperature drops to that of the environment, for they are incapable of arousing themselves from this state no matter what the external stimulus.

Hibernation as it is observed in the natural state takes place in the fall of the year and, except in the case of bats, is associated with the autumnal drop in environmental temperatures. However, the laboratory animals which normally hibernate in the fall may be induced to hibernate at any season of the year by maintaining them for a sufficient length of time under suitable conditions at an environmental temperature a few degrees above zero centigrade.^{16, 17, 18} It has been reported that certain animals "aestivate" during extremely dry or extremely wet seasons of the year. Thus some of the American ground squirrels¹⁹ and the Madagascan tenrec²⁰ aestivate during the summer months. We know of no body temperatures having been taken on these aestivating animals, but it seems reasonable to conclude that this is a modified form of hibernation in which the animal enters into some sort of torpid state with body temperature reduced to the cool temperature of its nest.

PREPARATION FOR THE HIBERNATING STATE

Fat and food storage. It has been known for many years that most animals which hibernate become enormously fat during the late summer months and enter hibernation in an obese condition. The golden hamster¹⁷ and the European hamster¹⁸ appear to be exceptions to this rule. Wade²¹ indicated that fat thirteen-lined ground squirrels tend to hibernate before thin ones when exposed to cold simultaneously. Johnson²² agreed and showed that fat animals hibernated longer. Animals such as the woodchuck depend completely on their stored fat for nourishment during the hibernating period, while ground squirrels store small amounts of food in spite of their obese condition.¹⁹ Hamsters, on the other hand, store enormous amounts of food in their burrows prior to hibernation.²³ Lyman²⁴ showed that there was a long delay in the onset of hibernation in hamsters that were exposed to cold but prevented from storing food. Thus, depending on the species, the storage of energy either as fat or fodder appears to influence the onset of hibernation. The species difference also applies to the denial of food, for Johnson²² showed that starvation

hastens hibernation in ground squirrels, and we have found the same to be true for woodchucks in our laboratory. On the other hand, as might be expected in an animal which depends on stored food, starvation never brings on hibernation in the hamster.²⁴

Wade²⁵ has reported that ground squirrels can gain weight very rapidly when given an unlimited supply of food and believes this is a characteristic of hibernators. Wilber and Musacchia²⁶ measured the lipids of liver and kidney in arctic ground squirrels trapped in July and September. The higher concentration of fatty acid, lipid phosphorus and phospholipid in specimens caught during July indicated a rapid fat turnover at this time, with a slower lipid metabolism as the animals approached the hibernation period in late September. Although it is a fruitful avenue of research, an exhaustive study of the relation of adipose tissue to hibernation has yet to be made, though the role of brown fat has been the object of some research and much speculation.

Recently Fawcett and Lyman²⁷ examined the depot fat in regard to its utilization during hibernation. Since many of the animal fats are solid at 5° C., the question whether solid or semisolid fat can be used by a hibernator is an interesting one. It was shown that exposure to cold (5° C.) caused hamsters to desaturate their depot fat, raising the iodine number about five points and thus lowering the melting point to some extent. A diet of 20% saturated fat caused the iodine number to decline 17 points in animals maintained at room temperature, but only 10 points in animals exposed to cold. Similarly, hamsters fed a diet rich in unsaturated fat showed a small increase of the iodine number at room temperature but a further increase of about five points was found in the animals exposed to a cold environment. Although the fat of animals at room temperature on a saturated fat diet was sufficiently saturated to be solid when tested *in vitro* at 5° C., the increased unsaturation of the fat in the animals exposed to cold permitted them to maintain their fat in a liquid or semiliquid state at the hibernating temperature of 5° C. It was further shown that the albino rat, which is incapable of hibernation, did not desaturate its fat when exposed to a low environmental temperature no matter what the diet (fig. 1). Thirteen-lined ground squirrels fed the same diet as hamsters had an iodine number of approximately 93 compared with a value of 88 in hamsters. The diet of saturated fat which increased the saturation in hamsters had no effect on the saturation of the fat of ground squirrels under the same conditions. The effect of cold on the ground squirrels' fat could not be determined because the ground squirrels entered hibernation too rapidly after exposure to cold.

In the case of the hamster, there is a long period after exposure to cold before the animal enters the hibernating state and during part of this time, at least, the fat is evidently being changed to a less saturated form. The depot fat of ground squirrels is more unsaturated at the outset and these animals enter hibernation in a much shorter time when exposed to cold. It is tempting to postulate that the delay in hibernation in the hamster has some relationship to the saturation and melting point of the fat. However, it has not been possible to shorten the period between exposure to cold and hibernation by feeding hamsters an unsaturated fat diet, nor to prevent or curtail hibernation by feeding them a saturated fat diet. Therefore, though the

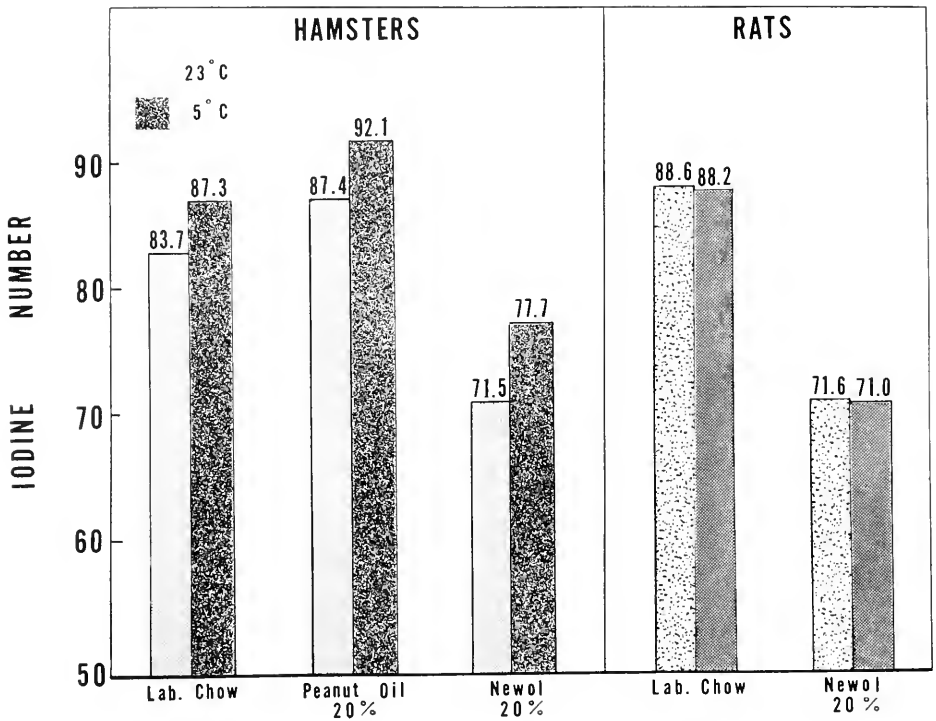


FIG. 1.—Effect of cold and diet on the saturation of fat in hamster and rat. “Newol” is the trade name of a saturated commercial cooking fat.

change of iodine number may well have physiological significance, its relationship to the onset of hibernation has yet to be demonstrated.

Brown fat. While considering the subject of fat, it is perhaps necessary to mention brown adipose tissue. Brown fat occurs in many parts of the body, but the portion located between the scapulae is often referred to as the “hibernating gland.” Rasmussen’s²⁸ careful work showed, however, that this tissue occurs in many animals that do not hibernate, including monkey, dog, cat and rat. Fontaine²⁹ has most recently defended this tissue as being of possible importance to the hibernating state. It has been shown that extracts of this tissue from woodchucks and ground squirrels³⁰ and hedgehogs³¹ injected into rats caused a lowering of metabolism. Such non-specific effects, as Wertheimer and Shapiro³² state in their review of animal fat, prove only that heterologous brown fat contains some substance that depresses metabolism. The lack of specificity is emphasized by the report of Nitschke and Maier³³ that extracts of lymphatic tissue from thymus, spleen and lymph nodes also caused a drop in metabolic rate. In this regard it should be emphasized that it is not known whether depression of metabolism is a cause or a result of the hibernating state. The observation that extracts of brown fat from woodchucks of unknown sex contained androgen³⁴ is of interest but does not seem to implicate the tissue in the problem of hibernation.

The stumbling block appears to be that the function of brown fat is very imper-

fectly understood and, therefore, its putative functions have an aura of mystery. Fawcett³⁵ has compared brown and white fat by a number of histochemical techniques and concluded that the tissues are qualitatively similar but that brown fat is a more active type of tissue, thus confirming the biochemical studies of Wertheimer,³⁶ Mirsky³⁷ and others. Other investigations have shown that brown fat reacts to insulin and other substances that influence carbohydrate metabolism.^{38, 39, 40, 41, 42} Thus evidence is accumulating that this tissue is indeed specialized for the storage of fat, but that the stored fat may be derived principally from carbohydrate rather than from ingested lipids. It has recently been shown that the storage and utilization of this fat is profoundly influenced by its nerve supply.⁴³ In conclusion, brown fat appears to be a specialized adipose tissue, and as such may have some significant function in animals such as hibernators which depend almost exclusively on stored fat for energy; but we know of no evidence which justifies calling this tissue the "hibernating gland."

The endocrines. Along with other preparations for the hibernating state it is generally accepted that the endocrine glands undergo an involution. This polyglandular involution has been observed by many investigators, and is the basis for one of the theories of hibernation which will be discussed at the end of this paper. Kayser^{18, 44} has ably reviewed the literature on this subject. Briefly, it may be said that at the time that mammals enter the hibernating state and during the hibernating period, the thyroid and adrenal appear histologically to be in a quiescent state. The ovaries and testes are atrophic before hibernation and remain so during the hibernating state, although in the last few days of hibernation the gonads of some mammals such as the woodchuck and ground squirrel show some signs of increased activity. (See *Growth*, page 96.^{45, 46, 47})

Involution of the thyroid and adrenal cortex upon exposure to cold is in direct contrast to the result obtained in many non-hibernating mammals in which cold causes a stimulation of the anterior pituitary, thyroid and adrenal cortex.^{48, 49, 50} Recently Deane and Lyman⁵¹ have re-examined the question of polyglandular involution during hibernation and have compared the reaction of the thyroid and adrenal cortex of the golden hamster and the white rat on exposure to 5° C. Keeping the animals at this temperature produced hyperplasia of the adrenal cortex and thyroid gland in rats but caused no detectable increase of activity of these glands in hamsters though the animals maintained a constant homeothermic body temperature (fig. 2). On the other hand, the pituitary-thyroid and pituitary-adrenal relationship has been shown to be normal in hamsters.^{52, 53, 54, 55, 56} It was therefore concluded that exposure to temperatures at which the animals normally hibernated was not sufficiently stressful to produce an "alarm reaction." Deane and Lyman⁵¹ emphasized that the decline in thyroid and adrenal activity which was considered by some to be intimately associated with hibernation actually took place in the summer time at the close of the breeding season^{47, 57, 58, 59, 60} which was often weeks or months before the time of hibernation. It thus appears to be a peculiarity of hibernators and some other wild species⁶¹ that they do not respond to the stress of moderate cold by enlargement and activity of thyroid and adrenal. This lack of response of endocrines so intimately concerned with metabolism must be permissive to the

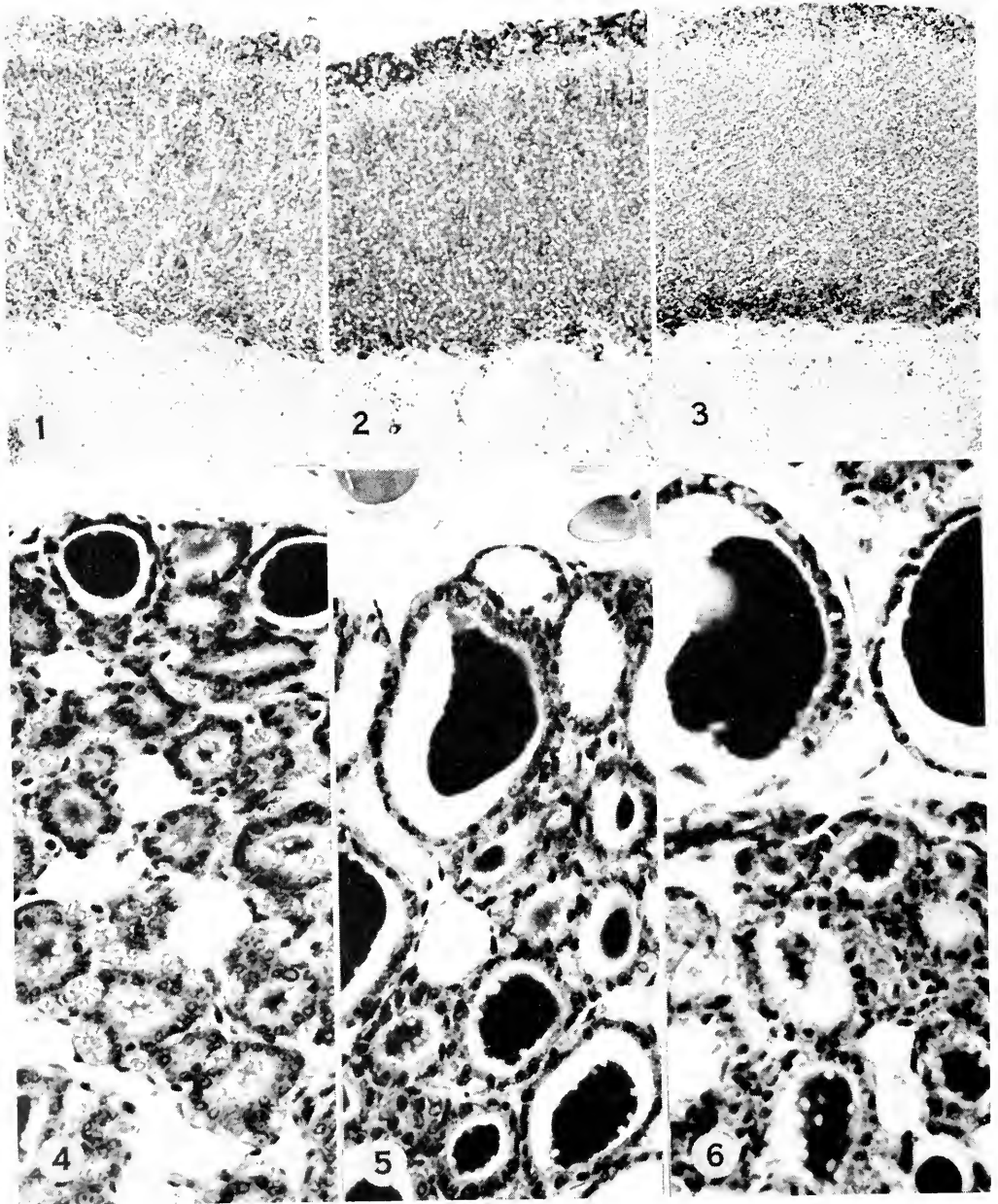


FIG. 2. Comparison of the thyroid and adrenal cortex of normal, cold-exposed and hibernating hamsters.

hibernating state, but the evidence is not convincing that the endocrines exert a dominant role in the onset of hibernation.[‡]

ENTERING HIBERNATION

Because of the difficulty of studying animals as they enter the hibernating state, this phase of the process of hibernation is little understood. Kayser¹⁵ has shown that several species of hibernators have a lower metabolic rate in the fall just before hibernation than they have in the spring. Dubois⁶² and Benedict and Lee⁶³ have pointed out that in animals such as the marmot and the woodchuck, the body temperature wavers for several days before the animal becomes completely dormant. These observations have been confirmed in our laboratories with woodchucks and ground squirrels, but a different sequence occurs in the golden hamster. This animal enters hibernation with one rapid decline in body temperature and takes about 8 hours to reach the environmental temperature of 5° C.¹⁷ The drop in body temperature, however, is not as rapid as that of a dead hamster of the same weight cooling in the hibernating position, which indicates that metabolism is slowing the cooling to some extent. Metabolic measurements show that the metabolism begins to decline at approximately the same time as the drop in body temperature, but the measurements are not sufficiently accurate to determine whether the temperature drop precedes or lags behind the drop in metabolic rate. As the animal enters hibernation, the decline in metabolic rate proceeds faster than the decline in body temperature and reaches its minimum several hours before the body temperature reaches the basic value of 5° C. (fig. 3). This may suggest

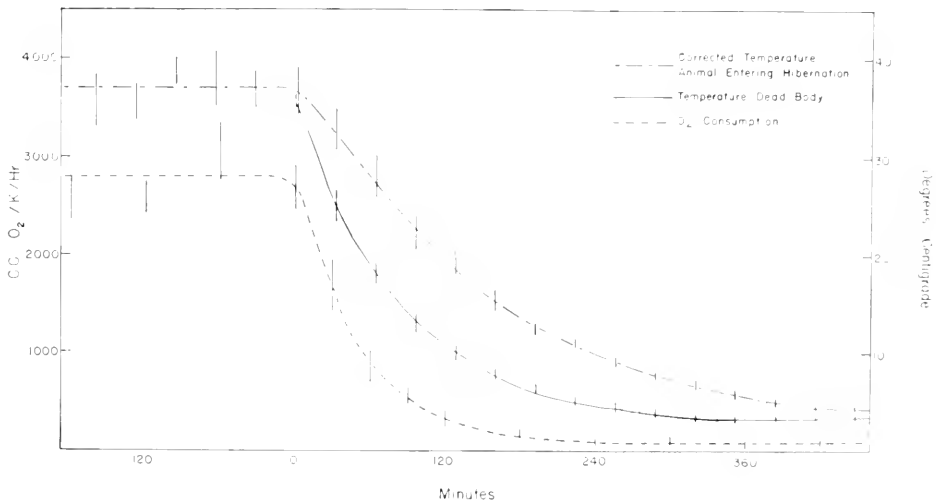


FIG. 3.—The upper line is an average of temperatures of the cheek pouch obtained from a single hamster entering hibernation on three separate occasions at an environmental temperature of 4° C. For comparison, the rate of temperature loss of two freshly killed hamsters is shown in the middle curve. The lower curve represents the oxygen consumption of the animal entering hibernation on the same three occasions. The vertical lines show the range of variation of the measurements.

‡ Recently, W. N. Holmes (*Endocrinology* 57: 409, 1955) presented confirmatory evidence by showing that there is actually a transitory rise in adrenal ascorbic acid in hamsters during the first three hours of exposure to cold. After this time, the ascorbic acid drops to a normal level, and remains at this level even during hibernation. There was no significant change in the weight of the adrenals during the total period.

that the decline in metabolic rate is the cause of the decline in body temperatures.⁸

IN HIBERNATION

Temperature. With the exception of bats, all hibernators curl in a tight ball during hibernation with the hair erect, the head beneath the tail and the tail usually curled around the body. This position permits a minimum of heat loss.

The body temperature of the hibernating animal is reported by Johnson,⁶⁴ Kayser⁶⁵ and others to be 0.5 to 3° C. above the environmental temperature, but Lyman¹⁷ found it was less than 0.5° C. above that of the environment in the golden hamster. Within a limited range, the body temperature passively follows the environmental temperature.^{63, 66, 67} Unless the environmental temperature changes very slowly there is always a lag between the body temperature and that of the environment, and many of the reported observations of body temperature either well above or below the environment are apparently due to a disregard of this lag.

Circulation. All investigators agree that the heart rate is remarkably slow during hibernation. Thus Hiebel and Kayser⁶⁸ report rates of two to three beats/minute in European ground squirrels and marmots; Suomalainen and Sarajas⁶⁹ observed a mean rate of 21/minute in the hedgehog, and Chatfield and Lyman⁷⁰ reported rates

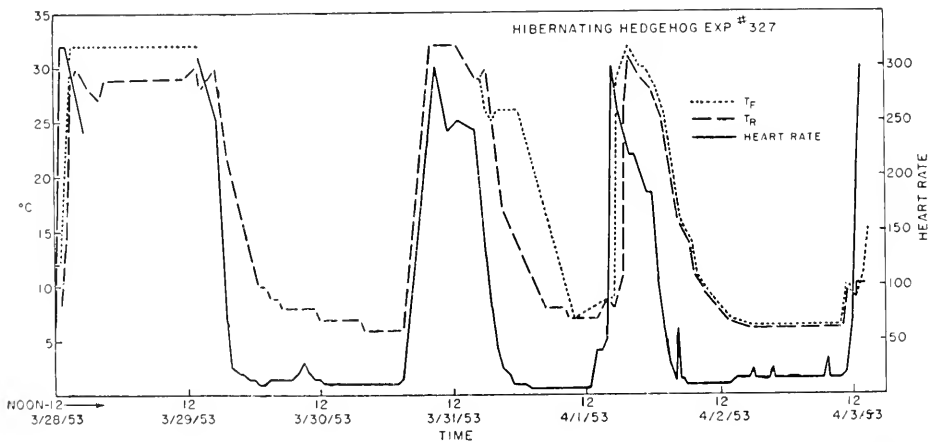


FIG. 4.—Hedgehog. The animal enters, remains in, and comes out of hibernation three and one half times in six days. T_F = anterior thermocouple; T_R = posterior thermocouple. (From Dawe and Morrison, 1955.)

⁸ A. R. Dawe and P. R. Morrison (*Am. Heart J.* 49: 367, 1955) have been able to study the EKG in hedgehogs and two species of ground squirrels (*Citellus parryi* and *Citellus franklini*) as the animals entered hibernation. One of their graphs indicates that there is a decline in heart rate prior to the drop in body temperature, but the senior author informs us that he is not convinced that this has been firmly established (fig. 4). They found the relationship of heart rate to body temperature very different in animals entering into and arousing from hibernation (fig. 5). When entering into hibernation, the heart rate falls rapidly with the decline in body temperature to about 20° C. At temperatures below this, the decline in heart rate in relation to body temperature is much slower. They point out that this indicates a hyperirritability of the heart of animals which hibernate compared to non-hibernating mammals. A previous study by H. S. S. Sarajas (*Acta Physiol. Scand.* 32: 28, 1954) also demonstrated a similar difference in the relationship of heart rate and temperature in hedgehogs waking from hibernation and in hedgehogs cooling in "artificial hibernation" after injection of insulin.

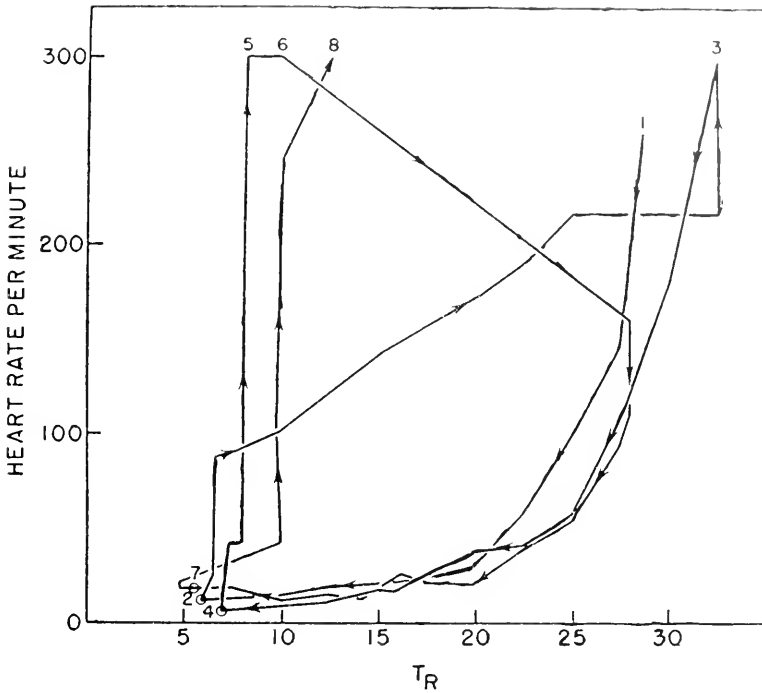


FIG. 5.—Hedgehog. Continuous record (Experiment 327) of heart rate and body temperature (T_R = rear skin temperature), showing hedgehog going into, in, and coming out of deep hibernation three times in five days. Times of events as indicated numerically on the graph are as follows: 1. 14:00—3/29/53. 2. 03:00—3/30/53, 03:00 3/31/53. 3. 12:00—3/31/53. 4. 01:00—13:00—4/1/53. 5. 17:00—4/1/53. 6. 18:00—4/2/53. 7. 06:00—4/2/53, 10:00—4 3, 53. 8. 12:00—4/3/53. (From Dawe and Morrison, 1955.)

varying from 4 to 21/minute in a group of 21 golden hamsters hibernating at 5°C ., with most hamsters having a rate of 8 to 9/minute. Later Lyman⁷¹ using chronically implanted electrodes decided that hamsters with the faster heart rates were either entering or waking from hibernation, and that the rate was never over 15 beats/minute in deep hibernation.

Auriculo-ventricular dissociation has been frequently described in hibernating animals or animals waking from hibernation.^{72, 73, 74, 75} It was also occasionally but not invariably observed by Chatfield and Lyman⁷⁰ (fig. 6). In this regard it is of interest that in non-hibernating animals whose body temperature has been lowered

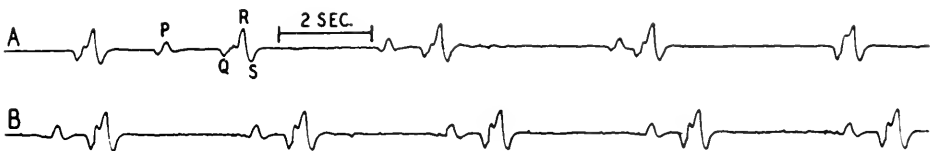


FIG. 6.—Ink writer record of the electrocardiogram of a hamster early during the process of arousal. Letters indicate components of electrocardiogram. A, showing A-V dissociation early during arousal; B, a few minutes later a normal rhythm has been attained.

artificially, auricular fibrillation, abnormally slow conduction and 2:1 and 3:1 A-V blocks have been reported.^{76, 77} This would indicate that of all the conducting mechanisms of the heart the auriculo-ventricular junctional tissue is the one most susceptible to the effects of cold.¹

No measurements have been made on the blood pressure of animals in hibernation, but measurements taken shortly after the start of arousal (see below) indicate that the blood pressure during hibernation must be very low.⁷⁹ The bright pink feet of the hibernating hamster indicate that the peripheral circulation is dilated, so that the heart is working against a low resistance. The extremely low blood pressure which probably exists in the hibernating state raises a number of interesting questions which should be answered by future research. For example, the activity of the kidney in deep hibernation and the problem of exchange of substances across the capillary wall in accordance with the Starling hypothesis deserve investigation.

Metabolism. Both the metabolic rate and the respiratory quotient of mammals in hibernation have been studied extensively. In general, it may be said that the metabolic rate of animals in hibernation is between $\frac{1}{30}$ and $\frac{1}{100}$ of the "resting" metabolic rate when the animal is in the homeothermic state. Kayser⁷⁸ has shown that, within the narrow temperature range of hibernation, the metabolic rate of the common dormouse increases with temperature according to Vant Hoff's law, but Popovic⁷⁹ failed to find this in the European ground squirrel.

Earlier workers reported questionably low respiratory quotients for animals in hibernation but the careful work of Benedict and Lee⁶³ and Kayser⁷⁸ established that in all mammals studied the respiratory quotient was very close to 0.7, indicating that the hibernator was using fat almost exclusively as the source of energy during the dormant period. In accordance with this, Carpenter⁸⁰ has shown that protein metabolism is greatly depressed in the woodchuck during hibernation although it is not altered qualitatively. The measurements of liver and kidney lipids in the arctic ground squirrel⁸¹ indicate that there is an increase of fat usage during hibernation when compared to the usage just prior to hibernation and confirm the conclusion that fat is the main source of energy during hibernation.

Benedict and Lee⁶³ measured the weight loss of woodchucks during the time the animals were actually hibernating and found that it amounted to 0.2 gms. per day per kilogram. Kayser⁸² has shown that the weight loss of European ground squirrels during the total period of hibernation is dependent on the length of time the animal is awake during that period. A single arousal with its following period of wakefulness uses more energy than many days of hibernation. Thus he showed that a ground squirrel which hibernated for 4,126 hours used a calculated total of 70 calories,

|| A. R. Dawe and P. R. Morrison (Am. Heart J. 49: 367, 1955) found that an Arctic ground squirrel showed paired beats during hibernation, and that both the Franklin ground squirrel and hedgehog had even bursts of heart beats. They also found that the T-P interval was greatly lengthened when the animal was in hibernation, and concluded that the primary cause for the slowing of the heart rate was the decrease in the automaticity of the SA node. Since the P-R interval showed lengthening second only to the T-P interval, they point out that the conduction time must also be slowed. The RS-T interval was slowed relatively the least, and this would indicate that repolarization was least affected by the hibernating state. H. S. S. Sarajas (Acta Physiol. Scand. 32: 28, 1954) reported very similar changes in the P-R interval with temperature in hedgehogs chilled after injection of insulin, and G. Björck and B. Johansson (Acta Physiol. Scand. 31: 257, 1955) further confirm this in normal hibernating hedgehogs.

while during the 330 hours that it was awake it used 579 calories.² Rasmussen and Rasmussen⁵³ reported a much greater proportional weight loss in hibernating woodchucks, as did Johnson⁶¹ in ground squirrels, but Kayser's contention that this was due to periodic arousals appears reasonable.

In spite of the depressed metabolic rate and extremely low body temperature, the hibernating mammal maintains a considerable degree of homeostasis. Early experiments indicated that exposure to sub-zero temperatures caused the animal to wake from the hibernating state, but Wyss⁵⁴ was the first to show that there was an increase in heat production in hibernating dormice when the environmental temperature was lowered to zero centigrade and that this higher heat production could continue for days without the animal waking from hibernation. Similar results have been reported for European ground squirrels by Kayser.¹⁵ Lyman¹⁷ confirmed this by taking simultaneous body temperatures and metabolic rates on hibernating golden hamsters. When the environmental temperature was lowered from 5° C. to 0° C. the animals maintained their body temperature at approximately 3° C. and tripled or quadrupled their metabolic rate. In some cases the animal remained in the hibernating state in spite of this increase in metabolic rate; in others, the animal awoke from hibernation and in a few cases, the animal was apparently unable to respond and died at the lower environmental temperature.

The nervous system. In deep hibernation, the cerebral cortex of hibernating animals shows no spontaneous electrical activity, but the species vary in the temperature at which electrical activity ceases. Thus the European ground squirrel⁵⁵ and the woodchuck⁵⁶ show spontaneous activity at a much lower temperature than the golden hamster⁵⁷ (figs. 7 and 8). Rohmer, *et al.*,⁵⁸ using the European ground squirrel, actually reported an "accident complexe," which we suspect is some sort of evoked potential, at central temperatures as low as 5° C. Brain temperatures were not given.

Other species differences exist in the sensitivity of central nervous structures to cold. Kahana, *et al.*,⁵⁹ who studied the round window response in the golden hamster

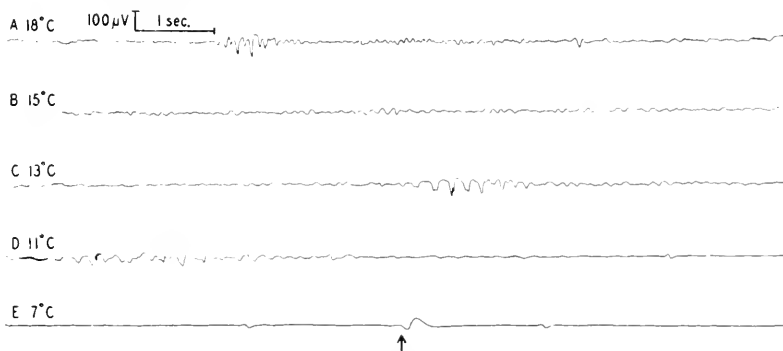


FIG. 7.—Electrocorticograms of a hibernating woodchuck at various cortical temperatures as indicated. The arrow in record E indicates a cortical response evoked by noise. Other deflections in record E are artefacts from EKG. Calibration in record A applies to all.

²E. F. Adolph and J. Richmond (J. Appl. Physiol. 8: 48, 1955) have calculated that the act of rewarming once every 11 days uses as much energy as the previous 10 days of hibernation in the thirteen-lined ground squirrel.

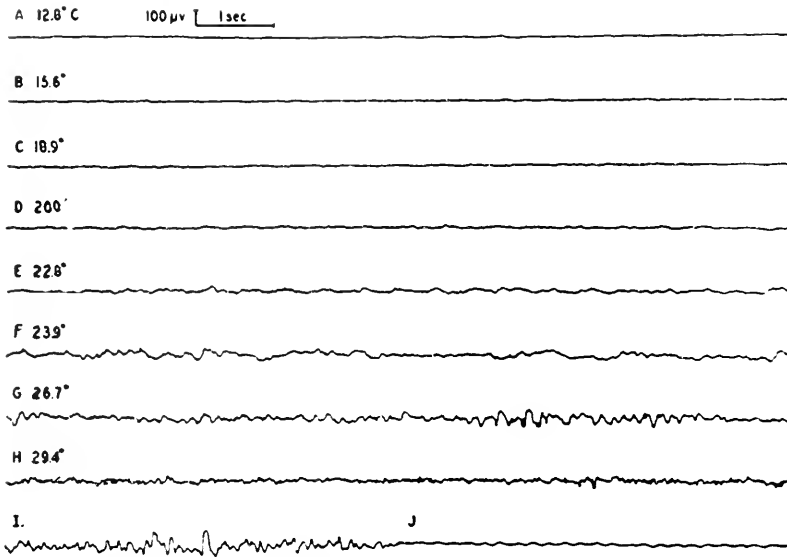


FIG. 8.—The electrocorticogram of an arousing hibernator at increasing cortical temperatures. Absence of definite electrical activity until record D, temperature 20°C . In records E, F, and G, increasing number of slow waves which eventually become grouped into bursts in record G. Onset of activation pattern in record H, at a still higher temperature. Record I, 1 minute after a subsequent injection of pentobarbital sodium. Note replacement of activation pattern with typical burst, similar to that seen in the colder unanaesthetized cortex in record G. J, 9 minutes after the injection. No cortical activity. Small waves are artefacts from the EKG.

as the animal was subjected to chilling, found that the neural component of this response, which presumably indicates conduction in the auditory nerve, could not be recorded below body temperatures of 18°C . That the golden hamster while hibernating is functionally deaf is borne out by the fact that we have never been able to arouse a hibernating hamster by the stimulus of sound. The woodchuck, on the other hand, which shows an evoked cortical potential in response to a sound stimulus at cortical temperatures as low as 7°C ,⁸⁶ probably does respond to environmental sound even in deep hibernation, as was indicated by the earlier researches of Benedict and Lee⁶³ and apparently the same holds true for the European ground squirrel⁶⁵ (fig. 7).

Acid-base balance. As early as 1896, Dubois⁶² reported a higher total CO_2 in the blood of hibernating marmots than in awake animals and used this finding as a basis for his autonarcosis theory of hibernation. This high total CO_2 has been confirmed many times.^{90, 91, 92, 93, 94, 95} Endres⁹¹ and Stormont, *et al.*⁹² reported a lower pH in the blood of hibernating ground squirrels. The high total CO_2 led to the conclusion that the respiratory center undergoes a loss of sensitivity during hibernation.^{44, 90, 91} This conclusion, however, disregards the well-established fact that the respiratory activity depends primarily on the CO_2 tension (pCO_2) and possibly the pH rather than the concentration of total CO_2 in the blood. Stormont, *et al.*⁹² were apparently the first to recognize this and calculated the pCO_2 of the blood of hibernating ground squirrels from pH and CO_2 determinations but admitted that

their CO_2 tensions were abnormally high. Using golden hamsters and ground squirrels, Lyman and Hastings⁹³ concluded that the total CO_2 was indeed higher during hibernation but the pCO_2 was lower. The total CO_2 in these experiments was not as high comparatively as that found by previous investigators but this was probably due to the extremely high total CO_2 in the anaesthetized controls. Bicarbonate and pH were essentially the same in hibernating and awake hamsters but the pH was somewhat lower in hibernating ground squirrels.

Recalculation of the data of Endres⁹¹ and of Stormont, *et al.*,⁹² showed that their results were similar except for the high blood CO_2 tension in Stormont's observations which disagreed with their own data on tissue gas pockets (table II). Lyman and Hastings⁹³ concluded that there was no evidence that the sensitivity of the respiratory center was depressed during hibernation, other than the observed fact that there was a larger range of variation in the acid-base balance of the hibernating animals. In a companion paper, Lyman⁷¹ showed that hibernating hamsters and ground squirrels responded to an increase of the inspired CO_2 by an increase in respiratory rate with about the same sensitivity as normal human beings (fig. 9). Ground squirrels, hamsters and probably all other hibernators have long periods of apnoea followed by several respirations. The apnoea could be abolished by high concentrations of CO_2 . Popovic⁹⁶ has shown that the hibernating European ground squirrel is also sensitive to lowering of the O_2 tension and that this animal responds to the lack of O_2 during hibernation in a manner similar to that of the animal when awake.

The work of Rasmussen,⁹⁰ Endres⁹⁷ and McBirnie, *et al.*,⁹⁵ indicates that the blood of the woodchuck is very similar to other mammalian bloods in its O_2 combining power. In view of the great reduction in the rate of O_2 consumption during hibernation, the gradient of O_2 tension from blood to tissues presumably would need to be much less than in active animals. The displacement of the O_2 dissociation curve to the left⁹⁸ as the result of lowered temperature means that a lower O_2 tension is required for the dissociation of oxyhemoglobin. However, since the percentage saturation of hemoglobin in venous blood of animals in hibernation is not essen-

TABLE II

AVERAGE DATA FOUND BY DIFFERENT AUTHORS FOR PLASMA pH AND CO_2

Species	No. of animals	Normal				Hibernation				
		pH	(CO_2) mM/l.	(HCO_3) mM/l.	pCO_2 mm.	pH	(CO_2) mM/l.	(HCO_3) mM/l.	pCO_2 mm.	
Hamsters ^a	13	7.39	37.3	35.5	60	15	7.44	42.4	40.0	32.4
Ground squirrels ^a	4 ^b	7.44	38.5	36.8	55.9	4	7.29	37.5	34.2	39.9
European hamsters ^c	2	7.32 ^d	24.2	22.8	46	2	7.22 ^d	33.8	30.4	42
Ground squirrels ^e	4	7.43	26.4	25.2	39	9	7.10 ^f	47.8	41.6	75
Ground squirrels ^g	3 ^g	7.30	42.0	39.5	83					49
			Tissue gas		66		Tissue gas			

^a Lyman and Hastings (1951).^b Awake in cold.^c Endres (1924).^d pH values were calculated.^e Stormont *et al.* (1939).^f pH values were corrected to temperature of the animal.^g Awake in cold, no water.

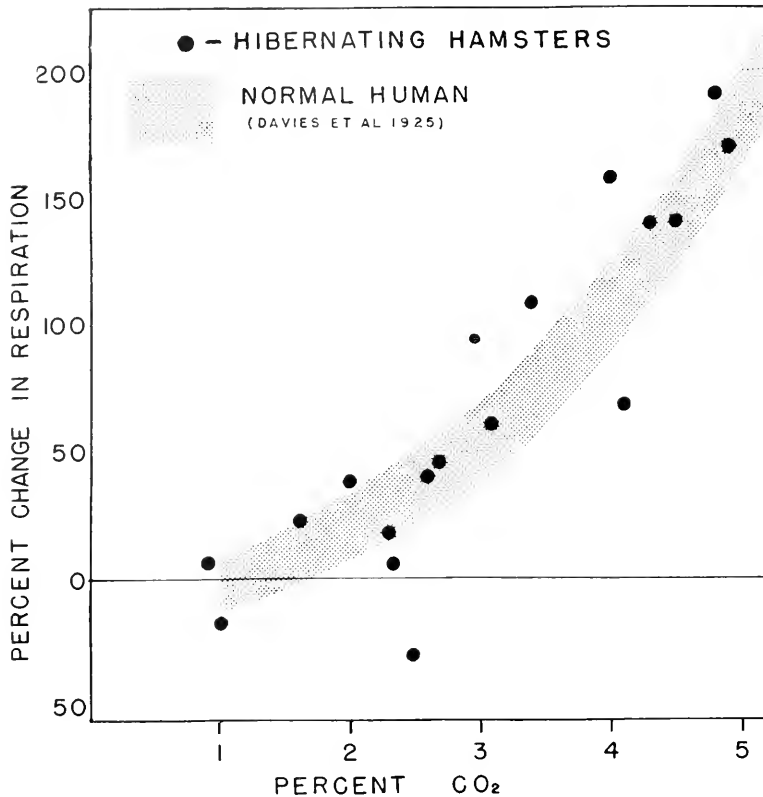


FIG. 9.—Effect of various concentrations of inspired CO_2 in a series of four hamsters compared to the response of man. Data on man from H. W. Davies, G. R. Brow, and C. A. L. Binger, *J. Exp. Med.* 41:37, 1925.

tially different from that of active animals,^{90, 95} it is apparent that this lowered O_2 tension gradient is adequate to supply the metabolic needs of the animal in hibernation. A low pH or a high pCO_2 would tend to correct the situation by shifting the dissociation curve to the right, and McBirnie, *et al.*,⁹⁵ suggest that this actually occurs in hibernation. Their evidence, however, is based only on observed high total CO_2 with no reported pCO_2 or pH.

Blood sugar. It has been reported for many species of hibernators that the blood sugar is low during the period of hibernation, but the degree of hypoglycemia apparently varies from species to species. Suomalainen⁹⁹ has reported a drop from 152–110 mg.% in the normal awake hedgehog to 85–49 mg.% in the hibernating animal. McBirnie, *et al.*,⁹⁵ have reported an average decline from 121 mg.% in the normal woodchuck to 66.8 mg.% in the hibernator, but Endres⁹⁷ found no decline in the hibernating marmot. On the other hand, Stuckey and Coco¹⁰⁰ and R. A. Lyman¹⁰¹ found a much smaller decline in hibernating thirteen-lined ground squirrels. Musacchia and Wilber⁸¹ found virtually no difference in the blood sugar content of hibernating and awake arctic ground squirrels, but showed that fasting animals

had low blood sugars and therefore concluded that prolonged fasting and hibernation were dissimilar processes, insofar as blood sugar was concerned. Lyman and Leduc¹⁰² found that the blood sugar in hibernating golden hamsters actually averaged slightly higher than the active animals and suggested that the reported differences in hibernators may be due to a difference in the habits of the animals involved. While ground squirrels and woodchucks are able to live in hibernation for a long time utilizing their fat as a source of energy, the hamster depends principally on stored food which he eats during periodic awakenings from hibernation. The periods of uninterrupted hibernation in woodchucks and ground squirrels are much longer than in hamsters, and the authors hypothesized that the blood sugar declines during the prolonged hibernating period. In a small series of European ground squirrels, Feinschmidt and Ferdmann¹⁰³ found that the blood sugar did decrease as hibernation progressed. If the results of Feinschmidt and Ferdmann are valid for the European ground squirrel, then arctic ground squirrels may have a different sugar metabolism during hibernation than the European species.⁵¹ In this regard it is interesting that the range of blood sugars found in hibernating woodchucks by McBirnie, *et al.*,⁹⁵ was from 36 to 140 mg.%, showing that this animal can tolerate a hypoglycemia in hibernation but that this condition is not universal. (Glycogen levels in the hibernator are discussed under *Metabolism*, page 108.)

Blood. The changes which may take place in the blood picture during hibernation are open to some dispute. Rasmussen¹⁰⁴ reviewed the previous literature, but failed to agree with earlier authors that the number of circulating red blood cells or the hemoglobin in the woodchuck decreased markedly during hibernation. Since there was no change in the size of the red blood cells, it may be assumed that the hematocrit also would have remained unchanged. On the other hand, he agreed with Dubois⁶² and others that the number of leucocytes was decreased by about one half in the hibernating state. Rasmussen and Rasmussen⁸³ found that the amount of blood relative to the body weight varied with the fatness of the animal. Obese woodchucks in the fall of the year, whether hibernating or not, had less blood relative to body weight than the leaner animals of late winter or early summer. On the other hand, McBirnie, *et al.*,⁹⁵ found a decrease in average hematocrit from 48.8 in hibernating woodchucks to 31.4 after hibernation.

Stuckey and Coco¹⁰⁰ reported a marked decrease in the erythrocyte count of hibernating thirteen-lined ground squirrels, with a concurrent drop in hemoglobin. The leucocyte count dropped only 2.3%. Using the same species, Svihla and Bowman⁹⁴ reported a decrease in the average blood volume of over 50% in hibernating ground squirrels, with an increase in erythrocyte count of from 6,700,000 in the awake animals to 12,000,000 in the hibernators. It is difficult to understand why the average hemoglobin determinations only increased from 15.6 in the active animals to 17.1 in the dormant ones under these conditions. The same criticism applies to the hematocrit values which were reported to "increase on an average of 0.09 to 0.14" (units not specified). In a later paper¹⁰⁵ the reported change in erythrocyte count was less dramatic—an average of 7,072,000 in awake animals to 9,042,000 in dormant ones. The hematocrits paralleled the erythrocyte counts, with an average of 37.26 in the awake to 50.15 in the dormant animals. Brace¹⁰⁶ found no lympho-

poiesis in the spleen and no erythropoiesis in the bone marrow of hibernating woodchucks.**

Many investigators who have worked on hibernation have noted that the blood of animals which are hibernating is very slow to clot and Svihla, *et al.*,¹⁰⁷ and Suomalainen and Lehto¹⁰⁸ have shown this to be the case in aestivating ground squirrels and hibernating hedgehogs respectively. Svihla, *et al.*,¹⁰⁹ indicated that the slowness in clotting is due to a lack of prothrombin, but they give no body temperatures or temperatures of the blood, so that the effects of blood temperature *per se* cannot be separated from the effects of lack of prothrombin.

Suomalainen⁹⁹ found a high serum magnesium in hibernating hedgehogs as did McBirnie, *et al.*,⁹⁵ with woodchucks, but the former reports a low serum potassium while the latter found it high during hibernation.†† Suomalainen⁹⁹ has reviewed the earlier contradictory results on serum calcium and reports very little difference in the awake and hibernating hedgehog. Kayser¹⁸ has thoroughly reviewed the changes in electrolytes and vitamins during hibernation and the interested reader is referred to his paper.

Growth. Growth during hibernation is greatly suppressed.^{110, 111} On the other hand, there must be some organ growth during the total hibernating period, for Foster¹⁷ showed that female ground squirrels breed less than five days after coming out of hibernation in the spring. Rasmussen¹² (confirmed by Brace¹⁰⁶) found slight changes in the gonads of the male woodchuck and greater changes⁴⁶ in the female before the end of hibernation. It is not clear, however, that these changes did not take place during the brief periods when the animal was awake. That the temperature of the tissues, rather than a lack of growth stimulus, is the factor which curtails growth during hibernation was shown by Lyman and Dempsey¹¹² by injecting testosterone into castrated, hibernating golden hamsters. In spite of the presence of a stimulus which caused enlargement of the seminal vesicles in the awake animals, there was no change in the animals which hibernated more than 7 days of the 10-day test period (fig. 10). Lyman and Fawcett¹¹³ have shown that homologous methylcholanthrene-induced sarcoma grafts in the hamster cheek pouch fail to grow during hibernation but are still viable and capable of proliferation when the body temperature returned to 37° C. after periods of hibernation lasting as long as 55 days (fig. 11). Courrier¹¹⁴ found that Wallerian degeneration proceeds more slowly in hibernating serotine bats than in awake animals. Smith and Grenan¹¹⁵ have shown that death from X-ray irradiation is greatly delayed in the woodchuck if the animal is hibernating, but that death occurs soon after the animal wakes from hibernation. This has been confirmed by Doull and Dubois¹¹⁶ using the ground squirrel.

** W. Z. Lidicker, Jr. and W. H. Davis (Proc. Soc. Exp. Biol. and Med. 89: 640, 1955) have confirmed earlier reports that the spleen in bats is greatly enlarged during hibernation and have correlated this with a marked decrease in the number of circulating erythrocytes. During the waking process, the engorged spleen released the erythrocytes into the blood stream. On the other hand, V. P. Rathis (Zeitschr. f. Biol. 106: 109, 1953) using the European hamster, and P. Suomalainen and T. Grauström (Exp. Cell Res., Suppl. 3: 335, 1955) using the golden hamster, both report an increase in total hemoglobin and in the number of circulating erythrocytes in animals during hibernation. They also show a similar, though not so large hemoco-concentration in animals exposed to cold but not in hibernation. It seems very probable that bats differ from other hibernators in this respect.

†† Recently, M. L. Riedesel and G. E. Folk, Jr. (Am. J. Physiol. 179: 665, 1954) report a 50% increase in serum magnesium after only 42 hours of hibernation in bats. Serum calcium level was slightly decreased and serum potassium remained unchanged.

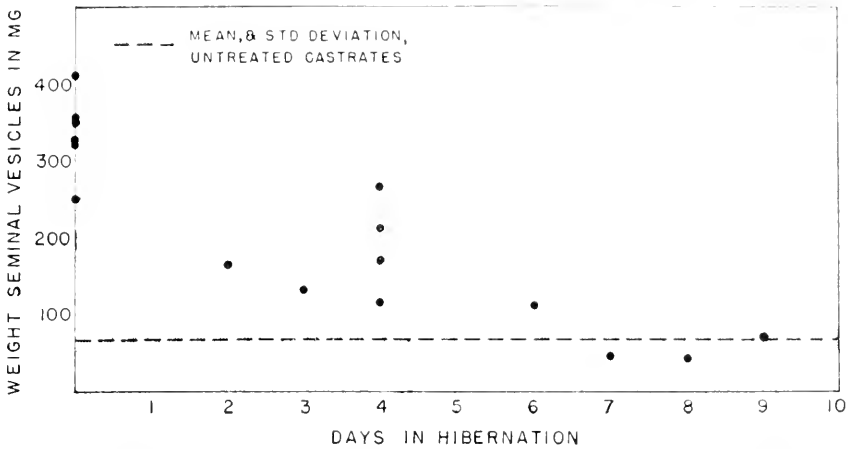


Fig. 10.—The weight of seminal vesicles of castrated hamsters which hibernated for various periods of time after injection of testosterone, compared with hamsters which did not hibernate. The mean and standard deviation of ten untreated castrates provides a base line.

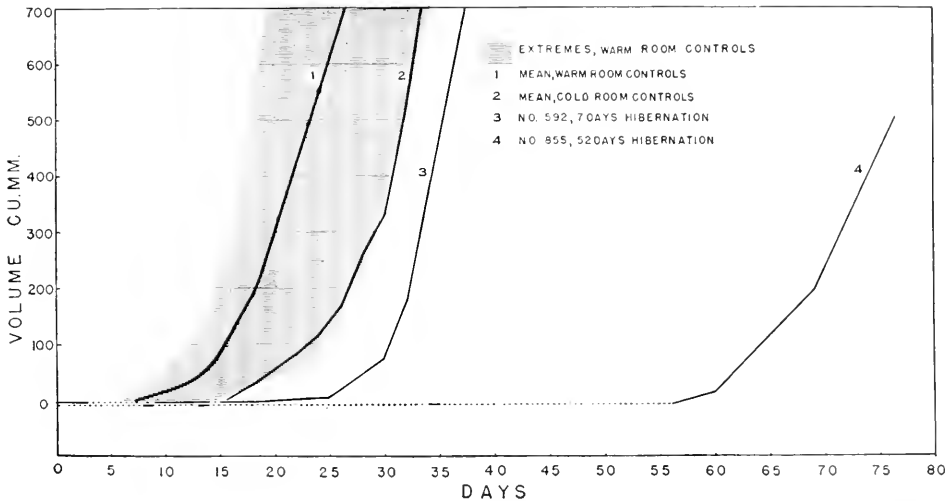


Fig. 11.—Effect of cold and hibernation on growth of tumor. In curves #3 and #4, the dotted horizontal portion of each line indicates when the animal was hibernating; the solid portion indicates when it was awake.

Since irradiation affects mitosis, it seems reasonable that the lack of cell division in hibernation temporarily protects the animal from injury.^{100‡‡}

Periodic wakings from hibernation. All reported laboratory experience indicates that the hibernating mammal wakes from time to time during the hibernating period and that these wakings are more frequent at the beginning and end of the

‡‡K. C. Brace (Blood 8:648, 1953) has indicated that the life span of the woodchuck erythrocyte is increased by exposure of the animal to cold, with a further increase of life span during hibernation, but the number of animals used is so small that the results are not conclusive.

total hibernating period. To our knowledge, the longest continuous period of hibernation was reported by Kayser⁷⁸ for a common dormouse which hibernated for 114 days. Although there is no way of knowing how long continuous hibernation may take place under completely natural conditions, it seems reasonable to assume that the usual period of continuous hibernation is much shorter than this record case. Observations in the laboratory indicate further that the lengths of continuous hibernation vary from species to species. Thus personal observation as well as the notes from other investigators¹⁸ indicate that animals such as ground squirrels and woodchucks probably hibernate continuously for several weeks at a time, while the golden hamster awakens as often as once a week and has been recorded as hibernating continuously for 21 days at the maximum.²⁴ The hamster is known to eat during the short periods when it is awake,²⁴ and it might be noted that gastric secretion continues in the woodchuck during hibernation.¹¹⁷

It is also true that species of hibernators vary in their sensitivity to external stimuli during hibernation, so that a stimulus which would start the waking process in one species will have no observable effects in another. For example, the hibernating marmot⁹⁷ will respond with uncoordinated muscular movements when poked with a sharp object but will not start the waking process, and Chao and Yeh¹¹⁸ reported that moving the quills of the hibernating hedgehog will cause the animal to first curl into a tighter ball and later relax after several deep respirations. On the other hand, such a stimulus in the golden hamster would immediately start the waking process. Our own experience with the hamster seems to indicate that the animals may be less sensitive to stimuli on one day than on the next, but the reason for this difference remains obscure.

The cause of the natural periodic awakening in the hibernating animal has yet to be conclusively explained, for any experimental modifications of the physiology of the hibernating animal may arouse it by the stimulation of pain alone. Thus Adler¹¹⁹ injected material which he believed contained thyroxin into hibernating hedgehogs and concluded that he had involved the thyroid glands in hibernation when the animals aroused from the hibernating state. Later Zondek¹²⁰ showed that warm physiological salt solution would produce the same result and Trendelenburg and Krayer¹²¹ pointed out that Adler's extract contained little thyroxin and other extracts containing little or no thyroxin had the same effect. Bruman¹²² was able to awaken hibernating common dormice by injecting atropine and thus considered that he had implicated the parasympathetic system in hibernation. Recently Svibla, *et al.*,¹⁰⁵ have aroused ground squirrels by injecting distilled water, using an intraperitoneal injection of mineral oil or air as a control and thus attribute hibernation to a lack of body water. Since even isotonic saline is painful when injected intramuscularly, it appears very unlikely that the animals did not experience pain which then started the process of arousal. More delicate and better controlled methods must be developed before the cause of spontaneous arousal is clarified. The reduction of some critical nutrient as a cause of waking has not been explored.

AROUSAL FROM HIBERNATION

General. The process of waking from hibernation is the most dramatic phase of the hibernating cycle, for it starts with an animal that is chilled and almost motion-

less and concludes about three hours later with a fully awake and active animal with a normal homeothermic body temperature. Although the waking process is slow to start, once well under way it evidently proceeds to completion in all species of hibernators. Certainly in the case of the golden hamster only death can stop the animal from struggling to regain its homeothermic temperature. All evidence shows that the arousal process is a highly coordinated physiological effort,^{18, 62, 63, 64, 70, 118, 123} in which the animal generates a maximum amount of heat in a minimum of time. In spite of this, many investigators have considered the early part of the waking process as physiologically the same as the deep hibernating state. In our opinion nothing could be further from the case, and failure to recognize this has resulted in many erroneous observations in the past.

The process of arousal consists of a series of precise physiological events which may be studied with relative ease, but this has been the most neglected phase of hibernation until recently. In general, the process is evidently similar in all terrestrial hibernators, but we will use the golden hamster as an example as we are most familiar with it.

When the hamster starts to arouse from hibernation at an environmental temperature of 5° C., the first visible change is a cessation of the periods of apnoea which are typical of deep hibernation. Except for fine, slow waving of the vibrissae, no other muscular movement can be seen. Within the first twenty minutes the respiratory rate may increase to 10, though the body temperature shows no measurable rise. At the end of 90 minutes, the cheek-pouch temperature has increased to about 10° C. and the respiration is up to 35 and regular.^{§§} If the animal is on its side, it begins to paw the air feebly, first with the front feet and later with the hind feet. After about two hours, the cheek-pouch temperature has increased to as much as 19° C., the respiration to 100, and the animal reacts reflexly to mild stimuli. Thirty minutes later the temperature is about 30° C., the respiration is above 100 and very irregular and the animal makes uncoordinated efforts to stand. From 150 to 190 minutes after the initial stimulus the cheek-pouch temperature rises to 36 or 37° C. During this time the animal is apt to shiver violently and its movements become increasingly better integrated. At the end of 190 to 210 minutes after being disturbed the animal is fully awake, with a temperature throughout the body of 37–38° C. (fig. 12).

Circulatory changes. If the temperature changes in the cranial and caudal parts of the body of the waking hamster are plotted against time, it may be seen that the cheek-pouch temperature shows a measurable rise about 20 minutes after the animal has been disturbed.¹⁷ Shortly after this, the temperature of the cranial portion of the body rises rapidly, reaching the homeothermic level in a total of about 170 minutes. The temperature of the caudal part of the body lags behind that of the cranial portion, so that near the mid-point of the waking process the cranial portion may be 20° C. warmer than the caudal¹²³ (fig. 13). Soon thereafter the temperature of the caudal portion rises rapidly and reaches the temperature of the cranial in a total time of about 200 minutes. Similar changes as arousal progresses have been de-

§§ The term "cheek pouch temperature" has evidently caused some confusion. (See E. F. Adolph and J. Richmond, *J. Appl. Physiol.*, 8: 48, 1955.) It was previously explained¹²³ that the cheek pouch of the hamster is so large that it reaches over the scapular region. Cheek pouch temperature is therefore the temperature of the thorax rather than that of the head.

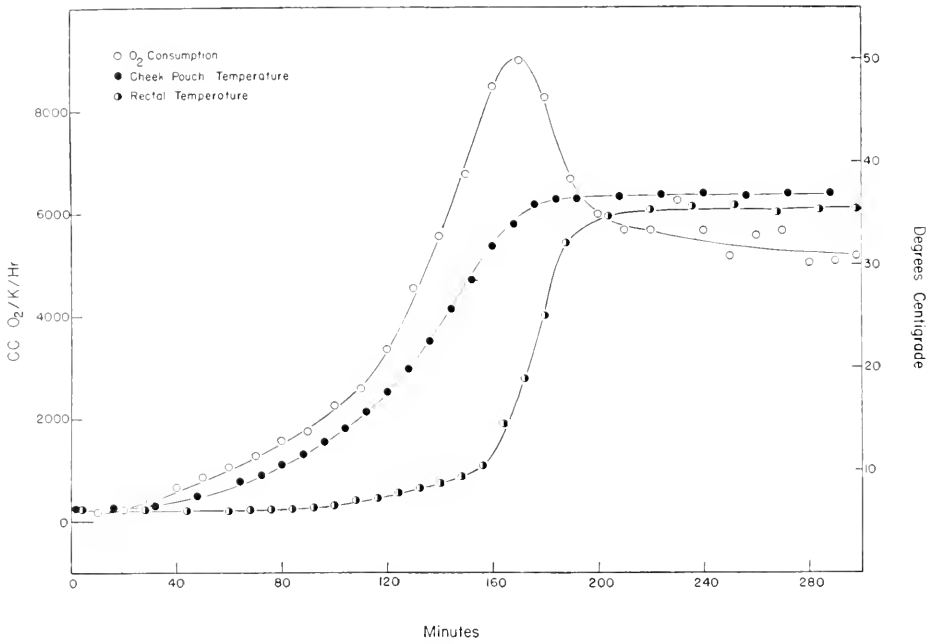


FIG. 12.—The open circles represent the average oxygen consumption of three hamsters waking from hibernation at an environmental temperature of 4° to 5° C. The closed circles represent the average cheek-pouch temperature, and the half-closed circles represent the rectal temperature of the same animals. Note that the rectal temperature rises more slowly than that of the cheek pouch.

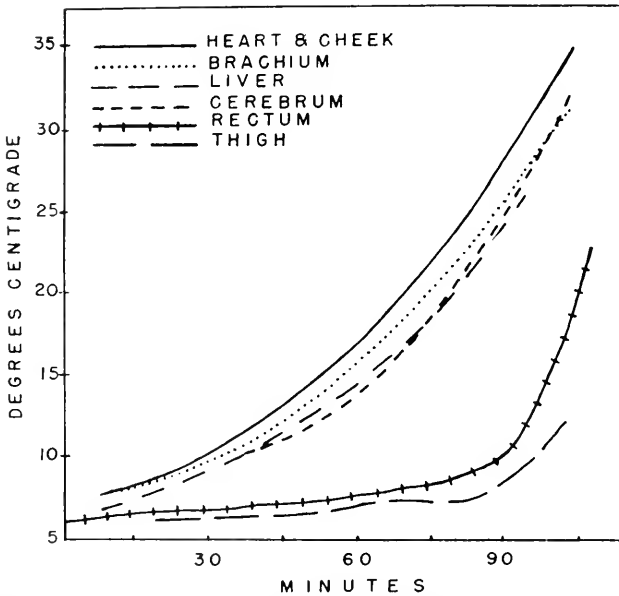


FIG. 13.—Temperatures in various parts of the body of a hamster waking from hibernation.

scribed in the marmot and woodchuck by Dubois⁶² and Benedict and Lee,⁶³ in the thirteen-lined ground squirrel by Johnson,²² and in the hedgehog by Chao and Yeh.¹¹⁸

That the temperature gradient during arousal is dependent on differential vasoconstriction was demonstrated by injecting a radio-opaque liquid (Thorotrast) into the hearts of both normal anaesthetized hamsters and animals waking from hibernation.¹²³ X-rays taken 3 seconds after the injection in normal anaesthetized animals clearly outlined the whole circulatory system. In contrast, only the cranial portion of the arterial system was outlined in the waking hibernator, and 30 seconds or more after the injection there were still only slight indications of circulation to the kidney, viscera and femoral arteries. There was no evidence in the X-rays of a possible arterial coarctation, nor could one be demonstrated by serial histological sections of the aorta (fig. 14).

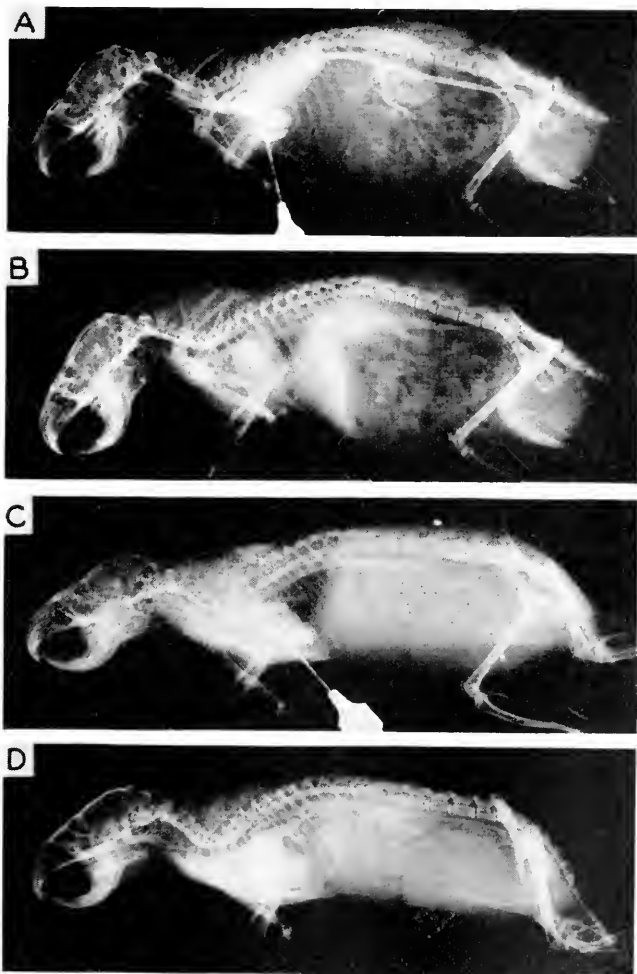


FIG. 14.—Thorotrast injected into heart of normal anaesthetized hamster and hamster waking from hibernation. A and B, normal hamster 2 and 9 seconds respectively after start of injection. C and D, waking hibernator 4 and 35 seconds after start of injection.

Marès using the European ground squirrel and Dubois⁶² using the marmot studied the circulation by injecting indigo carmine into the blood stream. Although they intended to demonstrate the circulatory conditions that existed during deep hibernation, it is evident that they were actually observing the distribution of blood during the waking process, for the procedure could not have failed to arouse the animals. Marès showed that the dye first appeared in the cranial portion of the body and Dubois noted that the dye seemed to be held back at the capillaries. Thus a differential vasoconstriction during arousal has been reported for three species of hibernators, and it seems reasonable that it occurs in the other species. Lyman and Chatfield¹²³ have also noted that the fore and hind feet of hamsters, which are quite pink during hibernation, turn pale soon after arousal begins, but the pigmented skin of most other species does not permit this observation.

The heart. As soon as a hibernating hamster is disturbed, any A-V dissociation which may be present immediately disappears, although the heart rate may not change⁷⁰ (fig. 6). Before any increase in temperature may be recorded, however, the heart rate increases and continues to do so until it reaches as much as 550 beats per minute as the hamster attains the 37° C. temperature.^{¶¶}

There has been much concern over the problem of whether the relationship of heart rate to temperature is a linear function. Knowlton and Starling¹²⁵ and Taylor¹²⁶ found a linear relationship in both cold and warm-blooded animals. Other investigators have obtained a nonlinear curve in the isolated and perfused hearts of frogs, rabbits, cats and dogs.^{127, 128, 129, 130} Some workers have reported an exponential curve in winter and a linear curve in summer in isolated frog hearts,^{131, 132} Endres, *et al.*,⁷⁴ obtained exponential curves from a marmot awakening from hibernation. Their experiment is open to criticism, however, on the ground that they placed thermocouples in the heart itself, which might have disturbed conduction, and the temperature range covered was small. Chatfield and Lyman,⁷⁰ in their studies of arousing hamsters, found that as body temperature rose the heart rate also increased, at first slowly, and then more rapidly, and eventually linearly. They ascribed the change in the slope of the curve to an increased effectiveness of sympathetico-adrenal activity, since Gellhorn¹³⁰ had shown that epinephrine increased the temperature coefficient of the heart (fig. 15).

When the logarithm of the heart rate of waking hamsters was plotted against the reciprocal of the absolute temperature a straight line was not obtained, showing that the phenomenon did not fit the Arrhenius equation which describes simple physico-chemical processes (fig. 16). This slowing was not due to vagal action, for a plot of the heart rate of completely atropinized animals was precisely similar to that of normal waking hamsters (fig. 17). The lack of vagal action was not due to inability of these nerves to function at low temperatures, for stimulation of the cut end of the right vagus at temperatures as low as 10° C. caused slowing of the heart. These findings may be compared with those of Badeer¹³³ who studied the influence of temperature on the denervated heart-lung preparation of the dog and found a linear relationship within the range of 25 to 38° C. He felt that the nonlinear relationships

¶¶ A. R. Dawe and P. R. Morrison (*Am. Heart J.* 49: 367, 1955), have confirmed the observation that the heart rate increases before an increase in temperature.

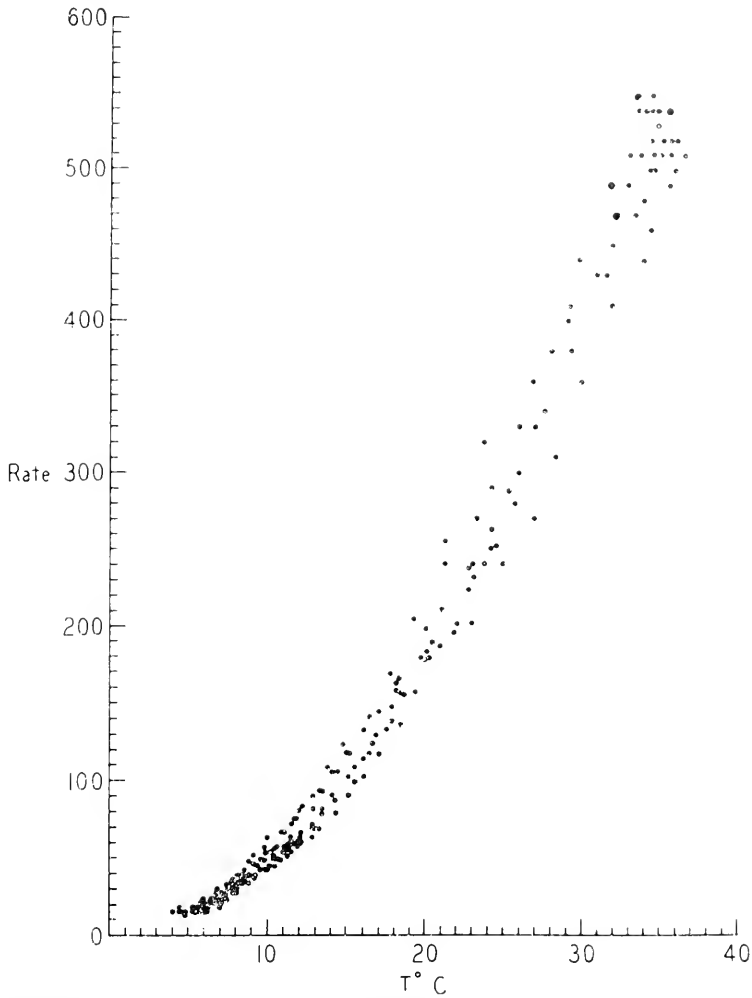


FIG. 15.—Composite graph of the heart rates/min. of 6 hamsters arousing from hibernation, plotted against their cheek-pouch temperatures. Linear coordinates.

reported in the literature were due to errors or omissions in experimental technique.¶

¶ The problem of the linear relationship of heart rate to temperature and also oxygen consumption to temperature in the waking hibernator has been explored further by C. Kayser, M. L. Rietsch and M. A. Lucot (*Arch. d. Sci. Physiologiques* 8: 155, 1955). Although their data do not fit the Arrhenius equation over the whole temperature range of 5° to 35° C., they feel that the plot actually falls into a series of three straight lines if the waking process is considered as three separate steps. With the complicated changes described below, which take place during arousal from hibernation, it would seem most unlikely that either heart rate or oxygen consumption would fit the Arrhenius equation. This conclusion is fortified by the observation of Dawe and Morrison (*Am. Heart J.* 49: 367, 1955) that the heart rate-body temperature function is totally different in arousal than during the process of entering hibernation.

G. Björck and B. Johansson (*Acta Physiol. Scand.* 34: 257, 1955), found a steeper slope in the plot of heart rate against temperature from 15° to 22°. It may be that one source of discrepancies in measurements of the relationships between heart rate and temperature in the arousing hibernator is that in some cases (Endres, *et al.*, Björck, *et al.*), the animal was warmed with heat from external sources. Since the control of circulation is such an important phase of the process of arousal, (see below), it seems likely that external heat might affect the heart rate.

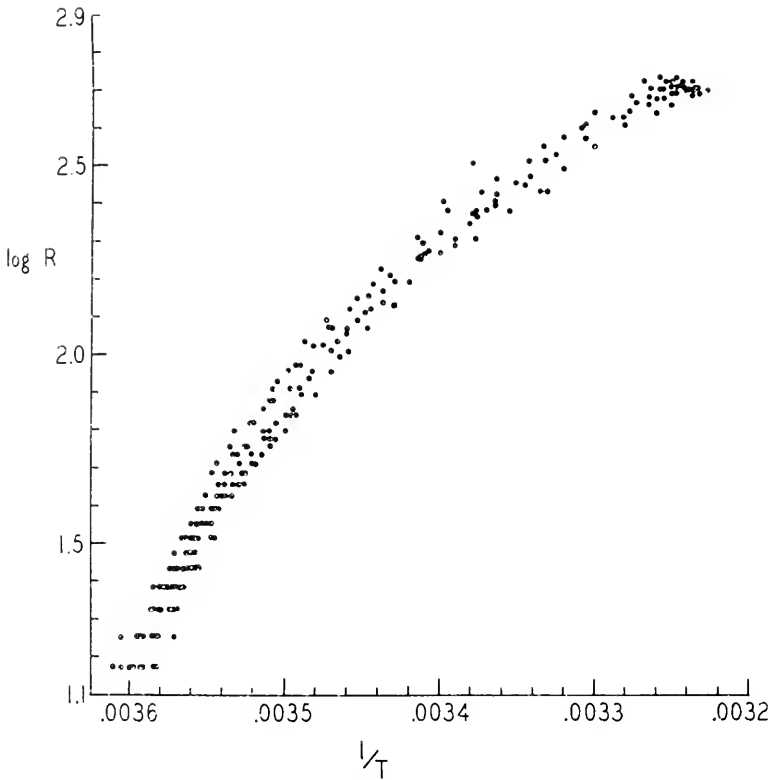


FIG. 16.—Data of figure 15 plotted to test for an exponential relationship. *Ordinate*: logarithm of the heart rate/min.; *abscissa*: reciprocal of the absolute body temperature.

Chatfield and Lyman,⁷⁰ who studied arousing hamsters electrocardiographically, found that the rate of conduction over the auricle and through the A-V node (as measured by the reciprocal of the P-R interval) also varied linearly with heart rate, while the duration of the QRS complex shortened markedly as heart rate increased (fig. 18). Accurate measurements of the latter were unfortunately precluded by the filtering condensers necessary for minimizing the muscle action potentials and slow base line shifts which occurred in the unanaesthetized animal. Another change in the electrocardiogram during arousal was an increase in the prominence of the T wave. This was interpreted as indicating the development of unequal rates of repolarization in the two ventricles. In the human, however, an increase in amplitude of the T waves is indicative of a high serum potassium¹³⁴ and the level of this ion in the serum of arousing hamsters might well be investigated. As the process of arousal progressed and the heart was beating more rapidly the P wave was seen to rise from a base line displaced by the T wave. Thus late in the process of arousal, when the heart attains extremely fast rates, another impulse must be transversing the auricles even before the ventricles are completely repolarized.

The rapid conduction of the cardiac impulse is interesting, for Walls¹³⁵ found that there were no Purkinje fibers in the atria or the right ventricle of the hamster heart, and only a limited number in the left ventricle. Thus the heart of the hamster

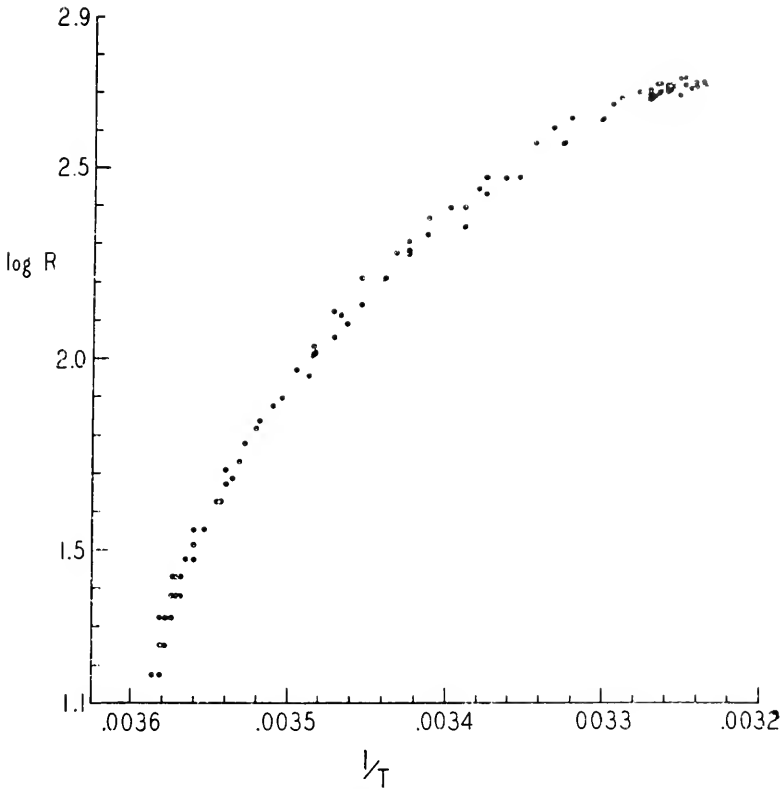


FIG. 17.—A plot of the heart rates and body temperatures of 3 completely atropinized hamsters.

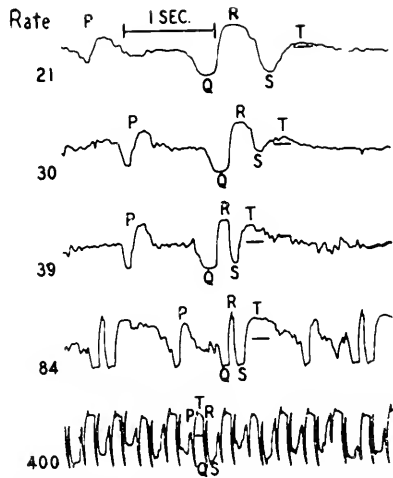


FIG. 18.—Ink writer record of electrocardiogram of a hamster during arousal showing increasing rate, shortening of P-R interval and duration of QRS complex, and changes in T wave. Letters indicate components of electrocardiogram. Horizontal line under T wave in each case is isoelectric line. Minor irregularities due to muscle action potentials.

can function at extremely high rates while having very little specialized conducting tissue.

Injection of the sympathetolytic drug veratrosine (veratramine glucoside) causes slowing of the heart of the waking hamster. On the other hand, the heart of the normal waking animal is not accelerated further by injected epinephrine. Therefore, Chatfield and Lyman⁷⁰ concluded that the heart during arousal was being driven at its maximum rate by the sympathetico-adrenal system.

Changes in blood pressure. Chatfield and Lyman⁷⁰ cannulated the carotid artery of hibernating hamsters and recorded the blood pressure with a condenser manometer. Although no record was made until at least 20 minutes after the hibernator was first disturbed, it was apparent that blood pressure was initially low. The initial measurement they obtained was on the order of 72/40, the mean pressure being 52 mm. of mercury. By the time the heart reached a rate of 100 beats per minute, the blood pressure reached a normal level. It was noted in these experiments that the pulse pressure increased transiently and then declined as the heart rate increased further (fig. 19).

Sources of heat. Dubois⁶² in his pioneer monograph on hibernation in the marmot concluded that the liver was the original source of heat in the waking animal, with contraction of the diaphragm being second in importance. Pembrey,¹³⁶ however, felt that shivering was of paramount importance, while Johnson⁷⁵ favored contraction of the heart and respiratory muscles in the ground squirrel. Lyman and Chat-

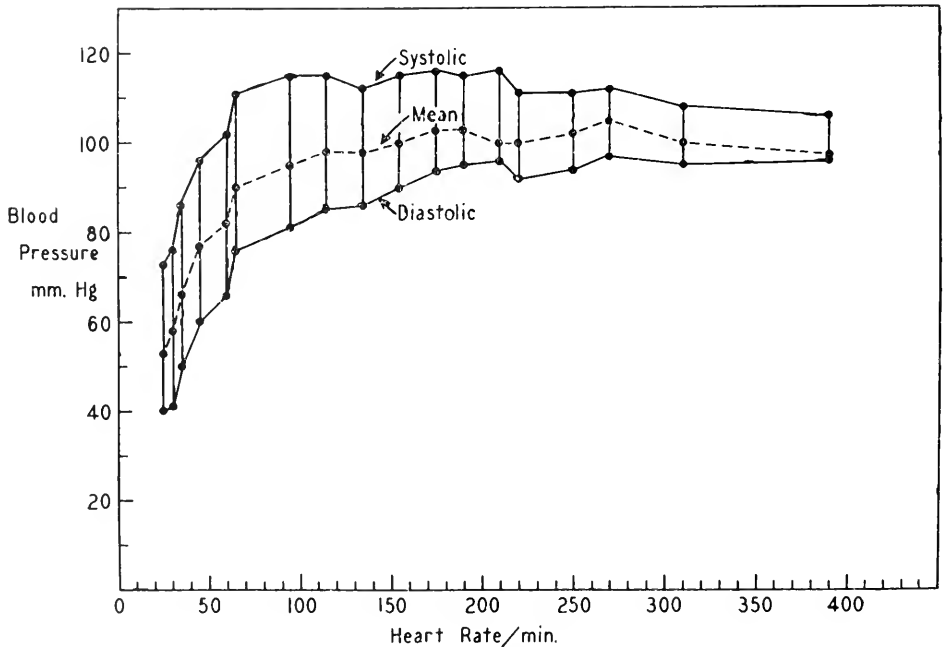


FIG. 19.—Systolic, diastolic, and mean blood pressures of a typical arousing animal plotted against heart rate, showing that blood pressure reaches high values long before the heart is beating at its maximal rate. Pressures were obtained with a condenser manometer which electrically integrates systolic and diastolic pressures to give the mean pressure.

field¹²³ were able to show that the cheek-pouch temperature of hamsters eviscerated during hibernation increased in the normal manner to 32° C., and in later experiments with improved technique, Lyman and Leduc¹⁰² demonstrated that the temperature could reach the homeothermic level of 37° C. in this preparation. It was concluded that the experimental procedures used to isolate the liver, on which Dubois based his hypothesis, caused acute portal stasis or pooling of the blood in the splanchnic bed,¹⁴⁷ and that it was these debilitating effects which impaired the warming process.

Although no muscle action potentials were detectable in the hibernating golden hamster except when the animal took a breath, action potentials appeared as soon as the animal started to awaken.¹²³ If the animal was momentarily touched or otherwise disturbed, these action potentials increased greatly in amplitude and so continued for a few seconds after the stimulus was removed (fig. 20). In spite of action potentials, there were no noticeable muscular movements at this time except for an occasional respiration. As the warming process progressed, the muscle action potentials increased in amplitude and frequency and finally became continuous. Unpublished observations indicate that very similar conditions obtain in the ground squirrel and woodchuck, except that occasional muscle action potentials may be recorded in deep hibernation as these animals make slight changes in their position.

Total curarization of the hibernating hamster caused marked slowing of the warming process, thus demonstrating the importance of the action of skeletal muscle. Remarkably enough, however, some warming took place even in the eviscerated, curarized animal. The heart may be implicated as an alternative source of heat for heart rate increased before any rise in body temperature, and slowing of the heart by anoxia (which acts reflexly via the vagus nerve) or by veratrosine, caused a drop in body temperature.⁷⁰ During arousal the heart of the hamster is beating very rapidly against a high peripheral resistance and, as Starling and Visscher¹³⁸ and

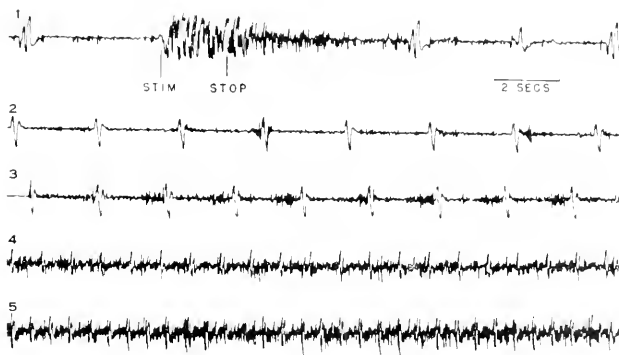


FIG. 20.—Ink writer records. Record no. 1 shows muscle action potentials and heart rate recorded 25 seconds after hibernating animal was first disturbed. At point marked "stim." animal was touched with a pencil. At "stop" the stimulus was removed. Note immediate increase of action potentials with stimulus and continued activity after stimulus was removed. Heart rate is 12 per minute. Records no. 2-5 illustrate the increasing heart rate and spontaneous muscle action potentials of animal waking from hibernation. Heart rates are 27, 33, 72 and 108 respectively.

Evans¹³⁹ showed, these are precisely the conditions under which the heart is mechanically most inefficient. This mechanical inefficiency implies that the cardiac musculature is dissipating a good deal of its energy in the form of heat. The contribution of the brain as a source of heat has not been studied, but it may be of some importance, as nervous tissue has a high metabolic rate and the waking hibernator is essentially a heart-lung-brain preparation.

The recording of muscle action potentials early during the process of arousal without any visible movement of the animal may reflect an increased muscular tonus. Fairfield¹⁷ in her studies of hypothermia in infant rats felt that this tensing was the cause of the augmentation of oxygen consumption in these animals. Indeed, Swift,¹⁴⁰ Dill and Forbes¹⁴¹ and Barbour, *et al.*,¹⁴² all reported that muscular rigidity and tonus were capable, in man and rats, of increasing the metabolic rate without actual shivering. There remains the possibility that heat may be produced in muscle without a corresponding amount of mechanical work being performed. Although the oxygen uptake of a tissue has usually been found to be proportional to the work done by that tissue, Loomis and Lipmann¹⁴³ have shown that these two phenomena are separable, since they found that the drug dinitrophenol uncouples the oxygen-consuming from the work-producing process. In the curarized arousing hibernator a similar reaction might take place, with some unknown factor in the situation playing a role similar to that of dinitrophenol, allowing the muscle to consume oxygen and produce heat without doing any mechanical work.

Metabolism. The oxygen consumption of arousing golden hamsters shows a measurable increase 20 minutes after the initial stimulus and continues to climb, at first slowly, then very sharply, until a maximum is reached in about 160 minutes.¹⁷ This maximum, which is a definite over-shoot, is followed by a decline to a more normal oxygen consumption. Though exaggerated by the annoyance which the awakened animal displays towards the thermocouples used for measurement of its cheek pouch and rectal temperatures, this over-shoot in oxygen consumption during the last hour of the waking process is also observed in animals unencumbered by such devices. The great burst of oxygen consumption which accompanies the process of arousal has been noted in all hibernators which have been studied, and Benedict and Lee⁶³ indicated that the peak of oxygen consumption during arousal in the woodchuck is greater than that obtained under conditions of maximum exertion in the normal animal.***

It is generally agreed that glycogen is the source of energy during arousal,^{144, 145} although Benedict and Lee,⁶³ from their RQ determinations, believed that the combustion of fat supplied the energy. Using chemical and histochemical methods, Lyman and Leduc¹⁰² have recently re-investigated this problem. Liver and muscle glycogen, which average the same in the awake and hibernating hamster, diminish rapidly during the process of arousal. Blood sugar remains essentially normal during this time, though it rises to hyperglycemic levels in a few animals. In animals

*** E. F. Adolph and J. Richmond (J. Appl. Physiol. 8: 48, 1955) found a close relationship between oxygen consumption and esophageal temperature in ground squirrels during waking, while colonic temperature showed little correlation. They concluded that the rate of oxygen consumption is largely governed by the temperature of some tissues near the esophagus. Maximal breath frequency occurred at a lower esophageal temperature than maximal oxygen consumption. This suggests to them that breath frequency was governed by the temperature of the lungs or chest more than by temperature of the brain.

denied the blood supply to the liver by evisceration and ligation, the blood sugar drops to hypoglycemic levels by the time the rostral portion of the animal has reached 37°, thus demonstrating the importance of the liver as a source of glycogen (fig. 21).

Glycogen in the heart muscle, which is high in hibernating animals, is still higher in animals denied food before hibernating. Macleod and Prendergast¹⁴⁶ reported a similar situation in the case of rats when the glycogen stores in the rest of the body were abnormally low. As in skeletal muscle, heart muscle glycogen declines during arousal. It is well established that glycogen of muscle is used directly as a source of energy¹⁴⁷ and the loss of glycogen in skeletal and heart muscle during arousal must indicate utilization by the muscles themselves. The extra store of glycogen in the heart of the hibernator re-emphasizes the importance of the heart in the process of arousal, for in the early stages of arousal the heart is capable of increasing its work load without depending on any exogenous source for energy (fig. 21).^{††}

Electrical activity in the central nervous system. According to Rohmer, *et al.*,⁸⁸ as the European ground squirrel woke from hibernation there was a progressive increase in the frequency of the waves in the electroencephalogram, while the amplitude (except for the "accident complexe") gradually increased up to a central temperature of 18°–21° C. and then diminished. Chatfield *et al.*,⁸⁷ recorded the electrocorticogram of the hamster during arousal and correlated it with the temperature of the cerebral cortex. Because of the steep temperature gradient between cranial and caudal parts of the body during arousal, head temperatures provide a much more valid comparison than body temperatures in such an experiment. It was found that no electrical activity could be detected until the cortical temperature had reached 19° to 21° C. At this temperature, slow, low voltage activity appeared, which was replaced at higher temperatures by spontaneous burst activity and, when the cortical temperature was about 29° C., by very fast frequency, low voltage discharges characteristic of an awake animal (fig. 8). Local strychninization of the cortex did not produce convulsive activity until the temperature had reached levels at which spontaneous activity would normally have appeared. Though the cortex appeared quiescent early in arousal, peripheral movement could still be elicited at temperatures as low as 12° C. by electrical stimulation of motor areas. Merzbacher¹⁴⁸ had previously reported that the cerebral cortex of torpid noctule bats was electrically excitable, but had given no temperatures. Chatfield, *et al.*,⁸⁷ were able to record a complex cortical response from stimulation of the sciatic nerve when the cortical temperature of chilled anaesthetized hamsters was as low as 9° C. (fig. 22). It was concluded that the brain stem reticular activating system was least resistant

^{†††} C. Kayser, M. L. Rietsch and M. A. Lucot (Arch. d. Sci. Physiologiques 8: 155, 1954) report a higher R.Q. in waking hamsters than in waking ground squirrels, and associate this with the extremely obese condition of the latter before entering hibernation, resulting in a greater use of fat during the waking process. They report a higher R.Q. in the ground squirrel toward the end of the hibernating season when the animal is extremely thin and consider that this indicates a use of protein. However, the interpretation of the source of energy by the measurement of respiratory quotient alone appears to us to be somewhat tenuous when the respiratory quotient is above 0.7. C. L. Dodgen and F. R. Blood (Am. J. Physiol. 179: 631, 1954) report a higher respiratory quotient in waking bats than during hibernation, and attribute this to the utilization of carbohydrate and/or protein as well as fat. From earlier observations (Federation Proc. 12: 34, 1953) of a very low supply of glycogen in the livers of hibernating bats, they emphasize that fat must play an important role.

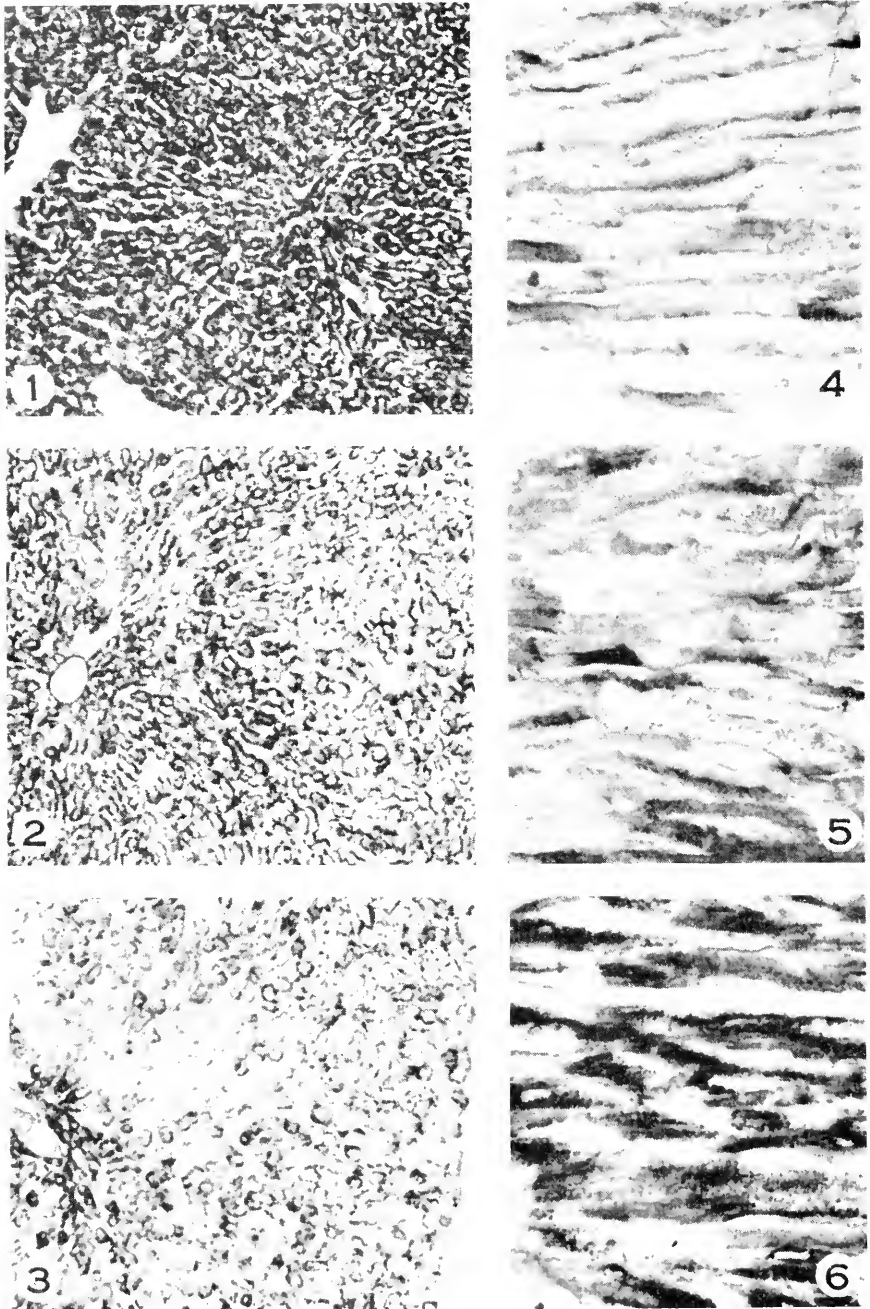


FIG. 21.—Photomicrographs of 5μ sections fixed in Rossman's fluid and stained with periodic acid-Schiff for the demonstration of glycogen. A Wratten B green filter was used to accentuate the fuchsian color and all pictures were printed to produce comparable color intensity.

1. Liver of a hibernating hamster fixed 43 minutes after the beginning of arousal. In this and in the two following figures the portal zone of the hepatic lobule is at the left, the central zone

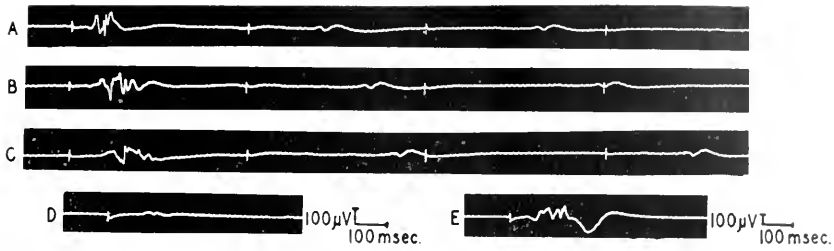


FIG. 22.—C.R.O. records of an evoked potential from strychninized cortex in an anaesthetized hamster on stimulation of the ipsilateral sciatic nerve showing the resistance of subcortical relays to cooling. A, B, and C show a series of shocks; D and E, responses to single shocks. Note that the cortical potential occurs only in response to the first shock of a series. Other deflections in records A, B, and C are artefacts from EKG. The latency of the response increases on cooling from 50 msec. in record A to 115 msec. in record D. Calibration after record D is for records A-D. Records E, after rewarming the animal. Latency 55 msec. Calibration after record E is for record E alone. Cortical temperatures: A = 11.7, B = 10.8, C = 9.4, D = 9.1, E = 13.6° C.

to cold because fast frequency, low voltage activity was the last to appear during arousal. The intralaminar thalamo-cortical circuits were felt to be second in order of resistance since spontaneous burst activity appeared before the fast frequency discharges. Spino-bulbo-thalamo-cortical relay systems and the cortex itself were judged to be more resistant than either of the preceding since the cortex was electrically excitable and an evoked potential could be obtained even at low temperatures.

In an extension of this investigation, Chatfield and Lyman¹⁴⁹ explored the cerebral hemispheres and brain stem of hamsters during the process of arousal and were able to record electrical activity at much lower temperatures than those at which spontaneous cortical activity occurred. The electrical activity recorded early in the process of arousal was obtained almost exclusively from components of the limbic system (fig. 23). These results strongly imply that it is the discharge of the limbic system, probably with the hypothalamus as a relay station, that initiates and co-

at the right. A large amount of glycogen is uniformly distributed throughout the lobule. Chemical determination of glycogen content: 40.9 mg./gm. $\times 125$.

2—Liver of the same animal fixed 34 minutes later when cheek-pouch temperature had risen to 11° C. Glycogen loss is evident throughout the lobule but is more pronounced in the central zone. Chemical determination of glycogen content: 33.2 mg./gm. $\times 125$.

3—Liver of the same animal fixed when arousal was complete, 130 minutes after the beginning of waking. Glycogen concentration has diminished further throughout the lobule. It is abundant only in those cells contiguous to the portal canal, almost absent in the middle zone of the lobule, and irregularly distributed in a mosaic pattern in the central zone. Chemical determination of glycogen content: 8.4 mg./gm. $\times 125$.

4—Cardiac muscle from a control hamster maintained in a warm environment. A very small amount of glycogen can be detected as fine dark granules deposited along one side of each muscle fiber. Chemical determination of glycogen content: 2.13 mg./gm. $\times 300$.

5—Cardiac muscle from a normal hibernating hamster. Glycogen is much more abundant than in control above. Chemical determination of glycogen content: 4.04 mg./gm. $\times 300$.

6—Cardiac muscle from a hibernating hamster which had been deprived of food. A heavy accumulation of glycogen is present throughout all muscle fibers. Chemical determination of glycogen content: 8.56 mg./gm. $\times 300$.

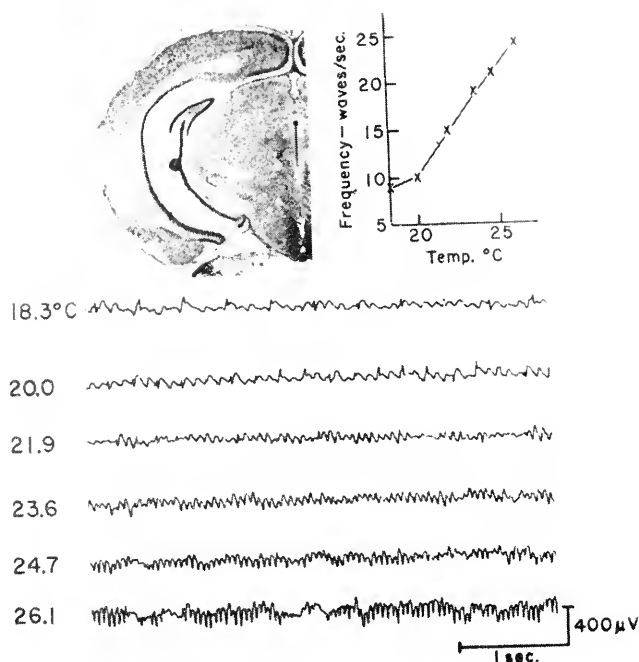


FIG. 23.—Burst activity recorded from one locus (black dot) in the hippocampus at the indicated cerebral temperatures. Inset shows a plot of the average frequency of the individual waves of the bursts as a function of temperature.

ordinates the process of arousal. Since the limbic system has been suggested as the neuroanatomical basis of emotions,^{150, 151} it is of some interest that the newly aroused hamster has been described as "being greatly enraged."¹⁷

The process of arousal as a coordinated physiological event. In summary, the following changes have been recorded in the hamster during arousal from hibernation: an increase in heart rate with the heart beating at a maximum rate for any given temperature; an abolition of A-V dissociation; an increase in the velocity of the cardiac impulse; a rise in blood pressure; a cutaneous vasoconstriction; a differential vasoconstriction between the fore and hind parts of the body; an activation of the somatic muscular system; an increase in oxygen consumption, and a glyco-genolysis. In other terrestrial hibernators in which some of the same measurements have been made, like conditions have been reported. All these phenomena, in the opinion of the authors, can be correlated in the hypothesis that the process of arousal is essentially a mass discharge of those parts of the nervous system which govern heat production and conservation, and which give rise to functional activity of the sympathetico-adrenal and somatic motor systems. Johnson⁷⁵ indicated that this activity was maximal, for further stimulation did not increase the speed of waking in the ground squirrel, and this is confirmed by the refractoriness of the heart to epinephrine in the waking hamster.⁷⁰

In spite of the importance of the sympathetico-adrenal system, neither the adrenal

cortex nor the medulla is essential for the process of arousal. Popovic and Vidovic¹⁵² have shown that the hibernating adrenalectomized European ground squirrel will waken normally when an adrenal graft in the anterior chamber of one eye has been quickly removed by enucleating the eye, and Lyman (unpublished) has observed normal arousal in hamsters that were adrenalectomized in the first five minutes of the waking period. Thus we cannot agree with Suomalainen and Herlevi¹⁵³ that the process of arousal necessarily induces the "alarm reaction" which is a chronic syndrome brought about by stress in which the adrenal cortex is of paramount importance.

PHYSIOLOGICAL SPECIALIZATIONS OF HIBERNATORS

It is to be expected that animals that hibernate should possess certain physiological modifications which are related directly or indirectly to hibernation. The ability of hibernators to desaturate their fat has already been described, but more data are necessary before this trait can be limited to hibernators alone.

As early as 1881, Horvath¹⁵ showed that hibernators could be chilled to much lower temperatures than animals that do not hibernate, and this has been confirmed many times. For example, woodchucks¹⁵⁴ and hamsters¹⁵⁵ can tolerate chilling to a colonic temperature of about 3° C. Animals that do not hibernate, on the other hand, rarely live when the rectal temperature drops below 15° C.¹⁵⁶ The study of the toleration of mammals to hypothermia has recently received great impetus because of the use of chilling in surgery, as well as the importance of exposure during war, but this problem is a subject in itself. It is of interest, however, that young mammals tolerate chilling to much lower temperatures than can adults of the same species.^{77, 156} As the young develop better temperature control, they are less able to tolerate hypothermia.¹⁵⁶ The relationship between these facts and the ability of hibernators to tolerate hypothermia has not been clarified.

The nerves of animals that do not hibernate cease to function at about 8° C., as Forbes and Ray¹⁵⁷ showed for the cat. On the other hand, the nerves must respond at lower temperatures in the hibernator, for there is a considerable degree of homeostasis at 5° C., and the hibernating animal responds to tactile stimuli, pain and temperature (see *In Hibernation*, page 88ff.). Greater resistance to cold in the peripheral nerves of hibernators was first pointed out by Tait,¹⁵⁸ who showed that a phrenic nerve-diaphragm preparation, as well as the excised heart from hibernating woodchucks and hedgehogs, exhibited activity at much lower temperatures than would be expected were the preparations from non-hibernating animals.

This problem was reinvestigated by Chatfield, *et al.*,¹⁵⁹ who compared the effects of cooling *in vitro* on the excised tibial nerves of adult golden hamsters and albino rats. They found that nerves from hamsters functioned down to an average temperature of 3.4° C., while nerves from rats ceased functioning at an average temperature of 9° C. (fig. 24). When nerves from rats were cooled, the action potential, conduction velocity and excitability decreased linearly with temperature. These variables decreased at a slower rate in hamster nerves and the action potential actually increased in amplitude in the early stages of cooling and then declined. Although some nerves of birds will adapt to the cold,¹⁶⁰ there was no indication of adaptation

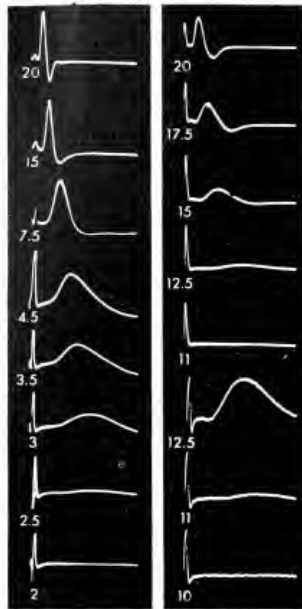


FIG. 24.—Action potentials of the tibial nerve of a hamster (on left) and of a rat (on right) at various temperatures. The action potentials of the hamster were all recorded at the same gain. The three potentials of the rat lowest in the figure were taken at a higher gain than had been previously used in this experiment. Note that even with high gain the action potential in the rat has disappeared at 10° C. while even with less gain the potential of the hamster is still perceptible at 2.5° C.

in this case, for the same results were obtained from the nerves of hibernating and nonhibernating hamsters. This resistance to cold appears to be a peculiarity of the peripheral nerves of all animals that hibernate, for similar results have been obtained in the woodchuck.¹²³ As was shown above (see *Metabolism*, page 91), the hibernating animal reacts with increased metabolism to environmental temperatures below 3° C. This is approximately the temperature below which the peripheral nerves will cease to function.

THEORIES OF HIBERNATION

The theories of hibernation can, perhaps, be divided into three categories though none can be separated completely from the others. The first theory is not widely accepted, but occasionally appears in the literature.¹⁶¹ According to this theory, mammals that hibernate are primitive, have poor temperature regulation and behave physiologically like the poikilothermic lower vertebrates. Hence, when it is cold, hibernators cannot resist the drop in temperature and therefore hibernate. We hope that those who have struggled thus far in this review will agree that the hibernators do not react to cold as do reptiles and amphibians. Except for the bats, hibernators can maintain the homeothermic state indefinitely,¹⁶² and all mammals in hibernation remain primed, as it were, so that an adequate stimulus may trigger the complex waking process. This is more a specialization of the homeothermic state than a reversion to the poikilothermic one.

The second general theory proposes that hibernation is brought about by a decrease or increase of a substance or substances which are ordinarily present in the mammalian body. From a theoretical point of view, the weakness of this concept is that it fails to explain how the animal can wake from hibernation. If an excess or lack of a given substance will cause an animal to hibernate, this imbalance must presumably be corrected before the animal can recover from the hibernating state. However, as has been mentioned above, the animal which has just entered hibernation actually wakes more readily than one which has hibernated for a long time.

Among those who have subscribed to this theory are Dworkin and Finney¹⁶³ who produced hypoglycemia in woodchucks by injecting large doses of insulin. These animals chilled when exposed to cold. Suomalainen,⁹⁹ having observed low blood sugar and high serum magnesium in hibernating hedgehogs, tried injecting a combination of insulin and a large amount of magnesium into animals exposed to cold. The hedgehogs chilled in a manner very similar to animals entering hibernation. In Dworkin and Finney's experiments the animals died without awaking, unless injected with glucose and moved to a warm environment; and Dische, *et al.*,¹⁶⁴ showed that injection of insulin would actually waken normal hibernating common dormice and European ground squirrels. In Suomalainen's experiments, glucose and calcium and a warm environment were necessary to produce recovery of the animal. As has been shown above, hypoglycemia is not a universal occurrence during hibernation, and we would attribute the chilling after insulin injection to hypoglycemic shock. The effect of large doses of magnesium could be due to the anaesthetic action of this metal, with calcium producing its well-known counteractive effect.

Dubois⁶² concluded that the marmot built up an excess of CO₂ in its burrow and that this caused the animal to hibernate. Actually it is conceivable that the excess or lack of some gas could cause hibernation, for the condition could be corrected quickly, even during hibernation. Recently Andjus¹⁶⁵ has chilled rats to 0° C. and Smith, *et al.*,¹⁶⁶ have chilled hamsters to -5° C. by exposing them to cold in airtight containers. The animals lack sufficient oxygen to fight against the cold and can be chilled to temperatures far below the lethal limit reported for these animals when given adequate oxygen.¹⁵⁶ However, if such a mechanism takes place during the entrance into hibernation, the methods of its functioning are completely unknown.

The endocrine glands should probably be included under the general theory of a hypo- or hyperfunction as the cause of hibernation. Much of the evidence implicating the endocrines has been based on a histological comparison of glands from awake and hibernating animals. We have mentioned that other seasonal changes in the endocrines have not always been differentiated from those which take place just as the animal hibernates, and the researcher is further hampered by not knowing which is the cause and which the effect of the hibernating state. As Kayser's review shows,²⁵ polyglandular involution exists in the hibernating animal and inactivity of the endocrines involved in metabolism is to be expected. Indeed, Foster, *et al.*,¹⁶⁷ showed that ground squirrels will not hibernate when fed thyroxin, or when injected with pituitary extract. The contrary result reported by Johnson and Hanawalt¹⁶⁸ may be due to the fact that they sterilized their thyroxin by boiling before injecting it. On the other hand the hibernating state as we have defined it has never been produced by removal of any one of the endocrine glands. Removal of the adrenals does not cause

hibernation in the ground squirrel;¹⁶⁷ in fact the presence of some adrenal tissue is considered necessary for hibernation,^{152, 169} and hypophysectomy results in eventual hypothermia and death.^{18, 167}

On the other hand, though the endocrines are probably not the basic cause of hibernation, they may well have profound influence in setting the stage for the final act, and it would be folly to disregard their possible importance in such obvious preparations as the building up of fat which takes place in many hibernators.

The third concept of hibernation implicates the central nervous system as the basic controlling mechanism. Prosser¹⁷⁰ has said that the outstanding fact of hibernation is the "turning down of the thermostat" and this concept postulates that the "thermostat" is in the central nervous system. It is well established that temperature control in non-hibernating mammals is regulated in the region of the hypothalamus and tegmentum and that chilling causes activity of the heat-conserving and heat-generating "centers." Assuming that hibernators have the same heat-regulating centers, these centers must undergo some change in sensitivity when the animal starts to hibernate. The nature or cause of this change is, of course, unknown, and there is actually no direct evidence implicating these centers in the process of entering hibernation. On the other hand, the central representation of the sympathetic nervous system appears to be definitely involved in waking from hibernation, and it seems reasonable to suspect that it is also of prime importance in the process of entering the hibernating state.

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DISCUSSION

Dr. Alan C. Burton: I would like to ask whether Dr. Lyman has verified the older observations about there being some abnormality in the hibernating animal of magnesium to potassium or sodium ratio, and whether he thinks this could be one tissue fact or biochemical fact which might be responsible for the different tissue states.

Dr. Lyman: I haven't done anything with magnesium. My personal feeling about any ionic imbalance is that if this is the situation it must have to be corrected the minute the animal starts to wake up from hibernation. I can't visualize exactly how this can occur. Even though there have been various reports concerning magnesium, some saying that it is high and some saying that it is normal, I haven't measured it.

Dr. Allen D. Keller: I would like to comment a little further about the presence of shivering in hibernation. The old literature never mentions whether there is shivering or not. I see you did demonstrate shivering in the woodchuck.

Dr. Lyman: During hibernation?

Dr. Keller: When they come out.

Dr. Lyman: They do shiver violently, particularly in the anterior end, as they warm to, say, 15 or 20° C. Also I have seen woodchucks shiver during hibernation every once in a while. They make gross movements and then seem to have a slight tremor. This is in the steady hibernating state. I have never seen that in hamsters. They curl up in a little ball and look completely dead until you pick them up, and then you see their whiskers move. That is all that seems to be happening.

Dr. E. Calkins: I was much interested in your observation on the serum epinephrine level during hibernation. Do you know what can be done to non-hibernators to stimulate their hibernation? We cannot change the diaphragms or tissues, but is it possible that administration of epinephrine competitors might enable non-hibernators to get along better at a low temperature? Do you know of anybody who has done that?

Dr. Lyman: Not as far as I know. It is worth while trying, certainly. The trouble is that there are side effects with almost any epinephrine competitors.

Dr. Calkins: If it could be administered without much difficulty, as I know you can with humans, and I am sure to animals, and prevent the epinephrine response to cold, you would avoid some of these complications that we are hearing about.

Dr. George E. Burch: I would like to know if you studied the peripheral circulation which shows the heart declines in rate as hibernation starts. Does the blood shift from the peripheral vessels into the body?

Also, you show that circulation in the lower half of the body is almost cut off. Does shivering take place in that part of the body without circulation, or is there any difference between the upper and lower halves of the body?

Dr. Lyman: In answer to the first question, we believe that during hibernation the animal's circulation is vasodilated. Evidence of this is not derived from measurement of peripheral blood flow or anything like that, but from the fact that in the hamster, the only hibernator having unpigmented feet, the feet are bright pink during the hibernating stage, but as soon as the animal starts to wake from hibernation the feet become pale, showing, we think, vasoconstriction. Observing these two things, we believe that the animal actually vasodilates as it goes into hibernation.

As far as shivering is concerned, the front end of the animal shivers violently, and it isn't until later when the blood supply is established that gross shivering begins in the posterior part. In fact, an animal coming out of hibernation in the cold room can crawl around with its front feet while it is still just dragging its rear end. So it really becomes well organized in the anterior portion before the posterior portion wakes up at all.

Dr. McMurrey: I would be interested in knowing how you get blood from an animal analyzed for epinephrine or glucose while he is hibernating.

Dr. Lyman: As far as the method I have used is concerned, the animal is really in the very start of the waking process when the drawing of the blood is completed. Actually, from an animal such as the woodchuck I think you could get blood from a chronically implanted cannula or something like that, but I have not attempted to do it. What I do is pick up the animal and within about a minute I am able to get blood from the heart by cardiac puncture. This animal isn't hibernating in the true sense, because it has already started to wake up. On the other hand, the slight changes which may take place in that short period should be smaller than the errors in blood analysis.

Dr. Henry Swan: I just wondered, Dr. Lyman, if you would discuss the state of the anesthesia in these animals during hibernation and during rewarming. I gather from your discussion that most of these animals when recovering from hibernation react to pain stimuli.

Dr. Lyman: We have been able to get and evoke cortical potential at temperatures as low as 7° C. by stimulating the peripheral sciatic nerve and picking up the potential at the cortex. So apparently these animals do react at temperatures as low as 7° C. The potential is a slow one, but it is there.

Dr. Chandler McC. Brooks: I think this brings up a point of some interest. If you remember the records of the action potentials in the hibernator at this crucial period of 7 to 4° C., although the amplitude of the action potential of the nerve was reduced, the area was greatly increased. Therefore, as far as the central nervous system was concerned, an impulse going in at that time would be many times as effective. It would act as a tetanus as far as the next synapse was concerned, compared to that of the normal. So that is an interesting thing—why the nerve of the hibernating animal retains this ability to be aroused when the other animals lost it so much earlier. It is an inherent characteristic of the nerve.

Dr. Lyman: In that regard, in the first record, I did point out that there were

places where it said "stimulated." At those places I simply poked the animal with a pencil. The action potential continued for many seconds after the poking. As you say, it continues for a long time.

Dr. Burton: This question is prompted by Dr. Burch's question. Since the remarkable x-ray pictures show that the blood doesn't go down to the femorals, wouldn't it be worth making a careful examination—perhaps you have—of the anatomy of the descending aorta to see if these animals don't have some specialized structure which they can clamp off. The diving mammals have that, as you know.

Dr. Lyman: I did that histologically, I am ashamed to say, in only one hamster. It takes quite a few serial sections. I looked through the sections and there is no evidence of a coarctation or anything like that in the one aorta. Of course, I could have missed a few sections.

Dr. R. K. Andjus: I should like to discuss Dr. Lyman's definition of hibernation. Dr. Lyman, in your definition you incorporate the fact that a hibernating animal is capable of spontaneous rewarming from hypothermia without the aid of external heat. I think that this is not at all specific for a hibernating animal. If one cools a non-hibernating mammal, a rat for instance, and leaves it at an ambient air temperature equal to its body temperature, the animal will rewarm spontaneously unless the body temperature was lowered below a critical level. If a rat is to be maintained at body temperatures higher than 15° , its effort to actively rewarm must be counteracted (by deep anesthesia for instance). Now the same is true to a greater extent for a hibernating animal. It is capable of rewarming spontaneously from much lower body temperatures. But this difference between the body temperatures from which hibernating and non-hibernating animals can rewarm spontaneously is only a quantitative difference, so to speak. A phenomenon that is more important for the characterization of hibernation and which represents a qualitative difference is the capability of hibernating mammals to remain dormant and not to start rewarming spontaneously in spite of the "aid of external heat." An unanesthetized rat will continue its efforts to rewarm spontaneously until the body temperature is forced close to the level below which cardiac arrest occurs. By contrast the hibernating ground squirrel although capable of spontaneous rewarming relaxes into hibernation and remains in hypothermia at body temperatures of 20° or more below the active body temperature.

Dr. Lyman: Unless it is disturbed, I think that is true. Possibly I should have emphasized that the nonhibernators, as you say, can rewarm spontaneously from 16° , or something like that, in the case of the rat, but they can't rewarm from 5° . I should also have said that my definition included the phrase, "a passive lowering of body temperature to that of the environment." In rats and other nonhibernators the only time they passively lower their body temperature to that of the environment is under anesthesia, drugs, and so on. They then are unable to warm without heat from external sources. As I said, it wasn't a very good definition.

RESUSCITATION AND RECOVERY OF HYPOTHERMIC, SUPERCOOLED AND FROZEN MAMMALS

R. K. ANDJUS, J. E. LOVELOCK AND A. U. SMITH

(This subject was presented to the Conference by a sound film entitled "Resuscitation of Hypothermic, Supercooled and Frozen Mammals" made at the National Institute for Medical Research, London, under the supervision of Drs. Andjus, Lovelock and Smith. There follows a summary of this film written by Dr. Smith and addenda furnished by Drs. Smith and Andjus. The floor discussion which took place after the showing of the film follows the addenda.)

SUMMARY OF SOUND FILM

The film opened with an introduction by Dr. Parkes. He said that previous films made in his Division on the effects of low temperatures dealt with individual cells and small pieces of tissues and with the use of glycerol to protect them from the otherwise fatal effects of freezing to, thawing from, and long-term preservation at -79°C ., the temperature of solid CO_2 . This work had naturally made him and his colleagues wonder how far they could go with the whole animal. Two very obvious questions had arisen: first, how to stop the heart beat and respiration by reducing body temperature in the range above zero in such a way as to permit reanimation on warming; second, what would happen to the animals when the body temperature fell below zero and when the body water began to crystallize out as ice. Dr. Parkes went on to say that at this point in their cogitations his staff had been fortunate in being joined by Dr. Andjus, of the University of Belgrade, who had been working on hypothermia in the rat at body temperatures down to zero, and who appeared to have solved the first of the two problems. Dr. Parkes then introduced Dr. Andjus who was doing an experiment.

Dr. Andjus then showed a normal rat being enclosed in a 2 litre jar which was placed in the refrigerator at $+2^{\circ}\text{C}$. He explained that the concentration of oxygen would gradually fall while the expired carbon dioxide would accumulate and that, as a result, the animal would itself cool down in the cold environment. Two hours later Dr. Andjus took the jar out of the refrigerator and demonstrated that the rat was unconscious and flaccid, and in a state of cold narcosis which could be used for surgical operations. The deep body temperature, recorded from a thermometer inserted into the colon, was 18°C . Electrocardiographic electrodes were then attached to record the heart beat both audibly and with a pen recorder. At 18°C . the rhythm was regular but very slow for the rat. The animal was then prepared for further cooling. It was thoroughly wetted with cold water and placed in a dish containing crushed ice, and the entire body, except for the nostrils, covered with crushed ice. Icy water was then added to fill the air spaces. Respirations were recorded by means of a rubber tube leading from the nostrils to a Marie tambour with a lever which wrote on a smoked drum. The last breath was taken when the deep body temperature had fallen just below 15°C . Soon after cessation of breathing the heart stopped beating. This was demonstrated by means of the electrocardiogram. The rat was left for one hour without breathing, with its deep body temperature dropping rapidly at first and then more gradually. By the end of the

hour the colonic temperature had dropped almost to the centigrade zero. Dr. Andjus said that rats kept without breathing and heart beats for one hour could regularly be reanimated by rewarming the heart with microwaves.

Dr. Parkes explained that the idea of using microwaves for heating the heart originated from Dr. Lovelock, and that the apparatus had been designed by him and built with the collaboration of Mr. Perkins in the Instrument Division of the Institute. He then introduced Dr. Lovelock who demonstrated his magnetron microwave generator which pours forth a flood of very high frequency radio waves. These radio waves can be projected in a narrow beam and are able to penetrate the skin of an animal and warm up its inner organs. Neither heat radiations nor longer radio waves share both of these properties.

Dr. Andjus then showed how the chilled rat was arranged in the path of the microwaves. The animal was placed under a wave-guide. The cardiac area was heated preferentially because it was situated under an appropriate sized hole in the wave-guide. The heart beat was thus re-established rapidly and before the extremities had become warmed. Artificial respiration was simultaneously administered by intermittent insufflation of air into the lungs through a rubber tube applied to the nostrils. An automatic air pump was used. After 15 minutes a regular cardiac rhythm had been re-established, heating and artificial respiration were discontinued and the animal took its first breath. The colonic temperature was still only 20° C. Movements of the head and tongue could soon be elicited. Respiration rapidly became regular as shown by kymographic tracings. The animal was then immersed in a bath of water at 40° C. to warm it further. Within 30 minutes it had regained muscular tone and the body temperature had reached approximately 30° C. The rat was then removed from the bath, dried, and put into an incubator running at 28° to 32° C. When placed on its back it turned over spontaneously. The animal was seen again one hour later sitting up and apparently in good condition. Dr. Parkes commented that Dr. Andjus had thus cleared the way for the second of the two problems previously mentioned, namely what would happen to an animal when the body temperature fell below zero. For this part of the work the golden hamster was selected because it had proved comparatively easy to handle in the range of body temperatures above zero. Dr. Parkes then introduced Dr. Smith who was at work in the laboratory. She showed a golden hamster in its cage, and explained that this rodent differs from the rat in being a potential hibernator. The experiments were, however, being carried on during the summer and the hamster was fully active. The hamsters were cooled by a method similar to that used for the rat. An animal was enclosed in a jar which was packed into ice. During the two hours which followed the deep body temperature fell by 20° C. to 18° C. The animal was then unconscious, and was transferred to a dish of crushed ice and surrounded by ice-cold water so that only its nose protruded. The body temperature fell still further but, unlike the rat, the hamster continued to breathe until it had cooled down to approximately +5° C. which is the lowest temperature usually reached in natural hibernation. During natural hibernation breathing and heart beats are slowed but never stop completely. The hamster was shown taking its last breath at the time when the colonic temperature was at +4.5° C. The deep body temperature was recorded continuously on a graph from a thermocouple inserted into the colon. The heart

stopped beating at $+3^{\circ}\text{C}$. The hamster was then immersed in a bath of propylene glycol kept at -5°C . in a deep freeze cabinet. The body was covered with blotting paper sodden with the fluid at this temperature, and in this way its trunk and limbs were weighted down and immersed completely while the mouth and nostrils were kept above fluid level. Within 10 minutes the deep body temperature reached -0.6°C . as shown on the graph, and then levelled off dropping almost imperceptibly during the course of the next hour while the hamster froze progressively. After freezing in this way for one hour the animal was so stiff that it could be placed on a rack supported only at the head and tail and would, in addition, uphold its own body weight as shown by placing a 100 g. weight on the abdomen. It felt woodlike to the touch and solid when knocked against the bench top.

The frozen hamster was rewarmed by deep and rapid heating of the whole body. This was done by means of a simple diathermy apparatus which had been built by Dr. Lovelock. The animal was placed between the diathermy electrodes and the high tension was switched on. Artificial respiration was given. The colonic temperature rose by about 1.0° to 2°C . per minute until it reached $+10^{\circ}\text{C}$. Diathermy was then stopped and the animal removed from the apparatus. Its heart was beating steadily. Soon normal breathing was resumed and artificial respiration discontinued. The nose, tongue, lips and paws which were deathly pale had become a rosy pink. When respiration was regular the animal was washed in cold water to get rid of the propylene glycol which was rather sticky. It was dried, and almost immediately started to shiver. In this way it rewarmed itself and no further external heating was needed. The hamster differs from the rat which does not shiver or rewarm spontaneously and requires to be heated artificially until its body temperature reaches the normal level. Even after that the rat needs to be kept in a warm place for several days. Within 40 minutes of being frozen stiff the hamster recovered muscular activity. The animal which had been filmed during freezing and resuscitation was by this time making vigorous efforts to turn itself over and finally, after a minute or two, it succeeded. Thereafter it recovered rapidly and was seen one hour later back in its cage, showing all the normal activity, including biting.

The same animal was photographed again eight weeks later. It was in very good health and showed no ill-effects. The only sign of damage due to freezing was loss of the pinnae of the ears which had become frostbitten because they had been knocked and bent when frozen stiff. Other parts of the surface of the body and extremities which had not been traumatized showed no signs of damage although abundant ice had been present in all the superficial tissues.

When cooled by the method shown, 75 per cent of hamsters became frozen. The other 25 per cent, treated in exactly the same way, became supercooled and the deep body temperature fell below zero, in some animals reaching -5°C . or below. In supercooled animals no ice formed anywhere in the body. They were much colder than the frozen animals but they remained soft and flabby and collapsed unless the whole trunk was supported. This was illustrated in the film by putting a supercooled hamster with a colonic temperature of -5.3°C . on the rack which supported it only at the head and tail. The animal promptly collapsed in a heap. It was then rewarmed with diathermy by exactly the same technique illustrated for the frozen animal. Artificial respiration was given as before. The heart rapidly resumed

beating and within 10 minutes was beating steadily so that the fur over the praecordium pulsated visibly. After 15 minutes the animal had resumed natural breathing. Within 30 minutes it had turned over and was shivering vigorously. Within one hour it was moving round actively, none the worse for its experiences, and actually looking for food. It recovered completely and showed no ill effects either within a few hours or many months after cooling to -5.3°C .

Dr. Smith said that the work on rats and hamsters shown in this film was only a beginning and was being extended to larger animals. Experiments on monkeys suggested that there were no fundamentally different problems and that there should be no insuperable difficulties either in cooling or in resuscitation. For instance, Dr. Andjus was shown cooling a monkey in a closed vessel in the refrigerator. After it had been narcotised with cold it was packed into crushed ice with only the nose protruding. The deep body temperature fell to $+5^{\circ}\text{C}$., and breathing was arrested for one hour. Dr. Andjus then revived the animal by means of diathermy and artificial respiration. Dr. Smith thought that this work might have important bearings on surgical anaesthesia, particularly in facilitating operations on the heart by allowing animals to be cooled to lower temperatures than were hitherto considered safe and thereby prolonging the period during which the heart could be excluded from the circulation and subjected to operations.

In order to keep animals in a state of suspended animation for periods longer than a few hours it would, on the other hand, undoubtedly be necessary to cool them to much lower temperatures in order to reach a state of biochemical and physical stability. Research along these lines had already begun at the National Institute for Medical Research.

ADDENDUM:

FORMATION OF ICE IN TISSUES

Furnished by A. U. SMITH

Dr. Lovelock and Dr. Smith have examined the distribution of ice within the body and have determined the amount of ice formed in the various tissues and individual organs as well as in the whole body of hamsters frozen for varying periods at -5°C . In hamsters frozen for 60 minutes as much as 90 per cent of the water in the skin, 60 per cent of the water in the brain, and 40 to 50 per cent of the total body water was often frozen. Nevertheless, animals frozen to this extent could be completely resuscitated. The proportion of early deaths and incomplete recoveries increased rapidly when the duration of freezing was prolonged so that no animal frozen for more than 70 minutes survived more than a few days. Breathing and heart beat were temporarily resumed in animals frozen for 170 minutes, but there was no sign of life in those rewarmed after freezing for three hours or more.

These results, as well as the effects of foetal development of freezing pregnant hamsters, will be reported in detail elsewhere (Smith, 1955 a & b; Lovelock and Smith, 1955). Hamsters resuscitated after freezing progressively at -5°C . do not necessarily become frostbitten in spite of the presence of abundant ice within the superficial tissues. Frostbite can, however, be induced by several methods which include bending the frozen extremities, and applying solid carbon dioxide at -79°C . to the frozen part (Smith, 1954).

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ADDENDUM:

CLOSED CONTAINER COOLING, AND OBSERVATIONS ON THE PHYSIOLOGY OF COOLING AND RESUSCITATION

Furnished by R. K. ANDJUS

I would like to give a summary of our recent work on hypothermia which was illustrated to a certain extent by the film presented to the Conference, particularly by the parts concerning the resuscitation of rats and monkeys after cooling to body temperatures of 0° and 5° C., respectively.

There are three main lines along which we have been working:

1. the method of inducing hypothermia by changing the concentration of natural respiratory gases;
2. the method of resuscitation from suspended animation in hypothermia by preferential heating of the cardiac area, and
3. the physiological study of the unanesthetized animal in hypothermia, including the study of prolonged and repeated hypothermia as well as of the period of recovery.

TECHNIQUE OF COOLING WITHIN A CLOSED CONTAINER

It was known since the work of Paul Bert and Raphaël Dubois toward the end of the last century that oxygen lack on the one hand and CO₂ excess on the other are capable of inducing hypothermia in animals. Giaja showed in 1940 and 1942 that rats, cats, and dogs can be cooled below 20° C. body temperature by reducing gradually the total barometric pressure in the container in which the animal is kept at ambient air temperatures of about 10° C. He showed also that rats become hypothermic when left in hermetically closed containers and reported of a case of spontaneous recovery after such cooling to a body temperature of 8.8° C. (Giaja, 1940 and 1941).

Rats cooled to about 15° C. by the closed container technique tolerate very well a variety of surgical operations and this method of cooling was suggested as a routine technique for inducing "physical anesthesia" (Giaja and Andjus, 1949, Andjus, 1950). The greatly increased resistance to oxygen deficiency in animals cooled by this technique made it particularly suitable for physical anesthesia in surgery where there was danger of asphyxia (hypophysectomy in rats, for instance) (Andjus, 1950).

The closed container technique of cooling enabled us (1) to cool safely animals

to body temperatures never reached before with subsequent recovery (about five degrees below the freezing point), and (2) to study the physiology of hypothermia unmasked by the effect of anesthetics or other drugs.

The routine technique as applied to rats consists of enclosing the animal in a jar of two liters capacity, placed in a refrigerator at 0° – 5° C. By the end of two hours the concentration of oxygen in the residual air decreases to 2 to 4 per cent, CO_2 to about 15 to 16 per cent, and the body temperature falls to between 15° and 20° C. (Andjus and Smith, 1955). A summary of some of the results of our investigations along these lines follows:

The critical and the lethal fall of oxygen tension. The heat production of an animal exposed to a gradual fall of oxygen tension does not begin to diminish until the oxygen tension has reached a critical level. The value of the "critical fall of oxygen tension" (CFOT) may amount to as much as 70 mm. Hg in different experimental conditions. It is influenced, for example, by the ambient temperature and the temperature of the environment to which the animal was adapted prior to the experiment (Gajda, 1953).

In order to answer the fundamental question whether the value of the critical fall of oxygen tension depends on the rate of oxygen consumption, we have done a series of experiments which will be described now. Four different groups of rats with different metabolic rates were exposed to a gradual decrease of oxygen tension and the values of their CFOT determined. Each experimental group had a group of control animals. In three of the experimental groups the oxygen consumption rate was very different from that of the corresponding control groups, due to thyroid feeding, hypophysectomy or different age (I, II, and IV, respectively, in fig. 1). It can be seen from fig. 1 that a highly significant difference in oxygen consumption rates between the experimental group and the corresponding group of controls was not necessarily accompanied by a significant difference in the values of CFOT (I and II in fig. 1). On the other hand, a highly significant difference in the values of CFOT was found between one of the experimental groups and its control group whose oxygen consumption rates were almost identical (III in fig. 1). It may be concluded that the value of the CFOT is not necessarily influenced by the rate of oxygen consumption. In other words, the critical fall of oxygen tension necessary to decrease the production of heat cannot be predicted on the basis of a simple oxygen uptake measurement.

The results in fig. 1 indicate, however, that although the value of CFOT is not necessarily determined by the rate of the total oxygen consumption, it may be influenced by the value of the "complementary" oxygen consumption. This complementary oxygen consumption, a result of the chemical thermoregulation, is calculated as *total oxygen consumption* (measured at a given ambient temperature) *minus basal oxygen consumption* (measured at 30 – 32° C.). In the experimental animals a greater complementary oxygen uptake was always accompanied by a smaller CFOT, and was independent of the value of the total oxygen uptake which might equal or differ from that of the controls (III and IV in fig. 1).

The results represented in fig. 2 show that the *lethal fall of oxygen tension* at a given ambient temperature is likewise not necessarily related to the rate of the total oxygen consumption. The values of the lethal fall of oxygen tension found

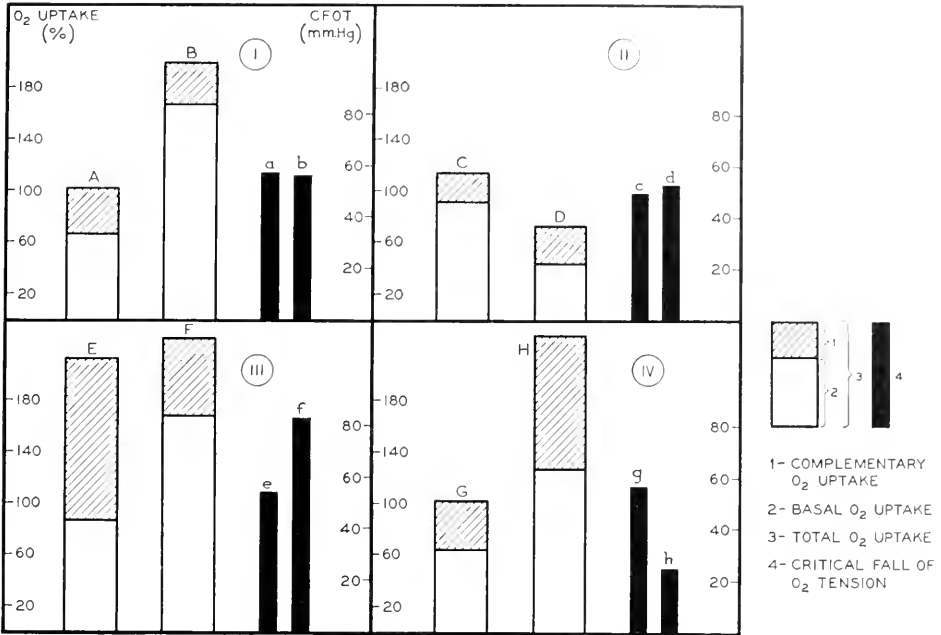


FIG. 1.—Critical fall of oxygen tension (black bars) in rats with different metabolic rates as measured by oxygen uptake (white and shaded blocks). A, a, C, c, E, e, G, and g are hypophysectomized rats; H and h are young rats weighing about 35 grams. I, II, and IV are results obtained at an ambient temperature of about 20° C.; III are results obtained at 10° C. The values for “Basal Oxygen Uptake” are obtained in preliminary measurements at 30–32° C. of ambient temperature. The fall of oxygen tension is calculated by taking 159 mm. Hg as the starting normal value. Each block or bar represents the average of 5 to 10 experiments.

for thyroid-fed rats are not very different from those found in the controls when the experiment is carried out at a high ambient temperature (30–32° C.), although it is precisely at this temperature that the difference in rates of oxygen consumption between the two groups of animals becomes most marked. On the other hand, when the rates of oxygen consumption of the two groups are almost identical, as it happens when they are both placed at a low environmental temperature (10° C.), the values of the *lethal fall of oxygen tension* differ greatly between the two groups.

While these results will not be discussed in detail, the significance of that portion of oxygen uptake that is involved in thermoregulation should be emphasized. The greater the proportion of the complementary heat production, the more easily will a small drop in oxygen tension be reflected in decreased consumption of oxygen and in the induction of hypothermia. When the share of heat which cannot be altered by the mechanisms of thermoregulation represents the major portion of the total heat production, it is then dangerous to attempt to decrease it by lowering the oxygen tension. When the latter type of animal is exposed to a gradual fall of oxygen tension, its heat production remains unaltered almost to the point of death. Its body temperature does not fall fast enough to permit hypothermia to exert its protective effect against oxygen lack. By contrast, when the major portion of the total heat production is a result of chemical thermoregulation, it can be readily de-

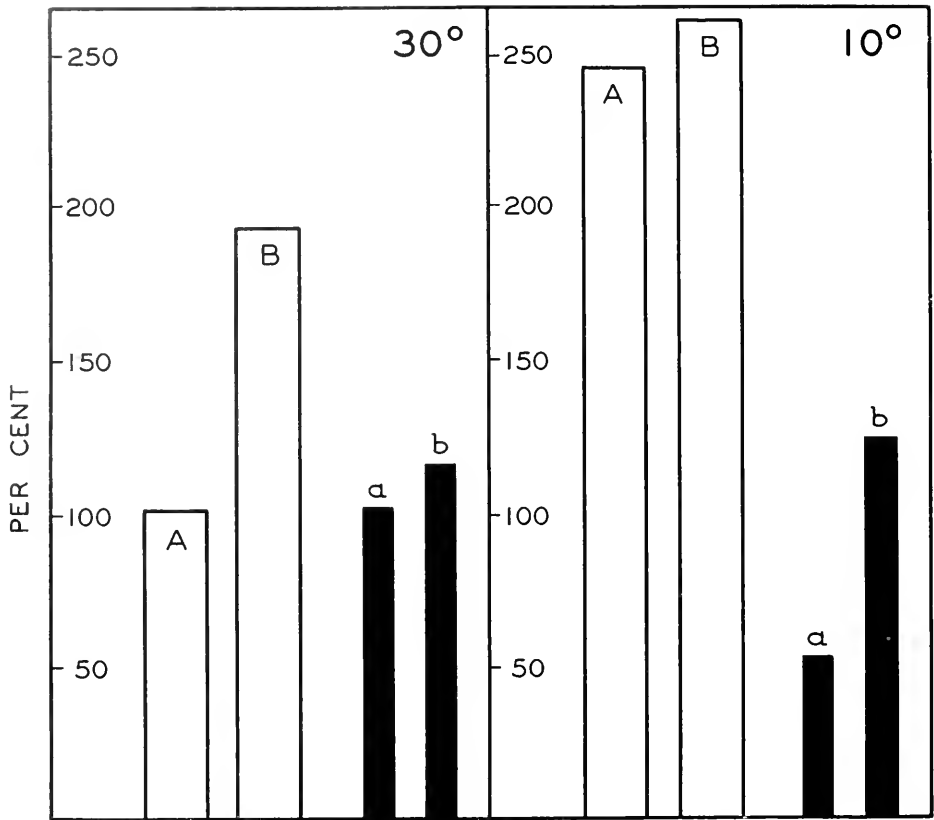


FIG. 2.—Lethal oxygen tension in normal and thyroid-fed rats at different ambient temperatures. White blocks show metabolic rate (per cent of normal BMR). Black bars show lethal oxygen tension (per cent of the value found in rats at 30° C.). A and a are normal rats; B and b are thyroid-fed rats. Values averaged from 5 experiments.

creased by a reduction of oxygen tension less significant in terms of vital functions. Hypothermia induced under these conditions reaches the protective temperature levels and helps the animal to survive further lowering of oxygen tension.

The severity of anoxia and the question of the "oxygen debt." It is important to stress the fact that it is the complementary heat production which is decreased by the lowered oxygen tension under the sealed container conditions described above. Hypothermia reaches deep levels long before the production of heat has fallen below the level of the BMR. The oxygen tension which is not sufficient to insure the complementary production of heat (i.e., the maintenance of the normal body temperature) is more than sufficient to maintain the vital oxidative processes. The fall of the metabolic rate below the level of the BMR takes place only when deep hypothermia is present, so that such a great depression of oxygen consumption is not the result of oxygen lack alone but of the lowered tissue temperature as well.

Although the oxygen uptake recorded on recovery can be significantly higher in

comparison to that recorded before the exposure, this cannot be interpreted as the result of an "oxygen debt." Rather, the increased oxygen uptake in the recovery phase is the result of the hypothermia itself. It is well known that hypothermia, if it does not reach a critical level, acts as "internal cold" and stimulates the metabolic rate to levels above that found in normothermic animals exposed to the same environmental temperature. In our experiments it is of significance that the consumption of oxygen during recovery showed no unusual increases in those cases where hypothermia alone was incapable of inducing a significant increase in heat production. This occurs in very young rats with deficient thermoregulation, or in thyroid-fed animals whose metabolic rates have already increased to a maximum. As might be expected, the stimulative effect of hypothermia on the production of heat is suppressed by oxygen lack. The appearance of hypothermia under hypoxic conditions is not accompanied by the rise of oxygen uptake which normally occurs in animals that are not deeply anesthetized.

The role of oxygen and carbon dioxide. During cooling by the closed container technique the animal is exposed not only to a gradual fall of oxygen tension, but also to the accumulation of CO₂. The effect of carbon dioxide can be illustrated by the experimental data summarized in table I. It can be seen that, at a high environmental temperature (30–32° C.) the accumulation of carbon dioxide is detrimental to the survival of the animal enclosed in the vessel. Contrariwise, at low ambient temperatures the rats survive better when carbon dioxide is allowed to accumulate. This effect can be partly explained by the fact that the accumulation of carbon dioxide slows down the oxygen uptake (fig. 3). This has two important effects on survival in the closed vessel. First, the production of heat is retarded (more than by hypoxia alone), and this promotes the appearance of hypothermia. Second, the fall of the partial pressure of oxygen is slowed down so that hypothermia has the time to reach deep levels before the oxygen tension in the tissues has

TABLE I

EFFECT OF CO₂ ACCUMULATION ON SURVIVAL IN CLOSED VESSELS KEPT AT DIFFERENT AMBIENT TEMPERATURES

(At 30–33° C., rats survived when CO₂ was continuously absorbed on KOH, but died when the experiment was repeated without absorbing the expired CO₂. In the lower temperature range the reverse was observed.)

No. of rat	Air temp. ° C.	Time spent in vessel min.	CO ₂ allowed to accumulate	CO ₂ eliminated continuously
1.....	30.6°	60	dead	alive
2.....	32.0°	82	dead	alive
3.....	32.3°	55	dead	alive
4.....	32.5°	70	dead	alive
5.....	22.5°	86	alive	dead
6.....	22.5°	117	alive	dead
7.....	17.2°	90	alive	dead
8.....	17.0°	113	alive	dead
9.....	14.0°	115	alive	dead
10.....	8.7°	107	alive	dead

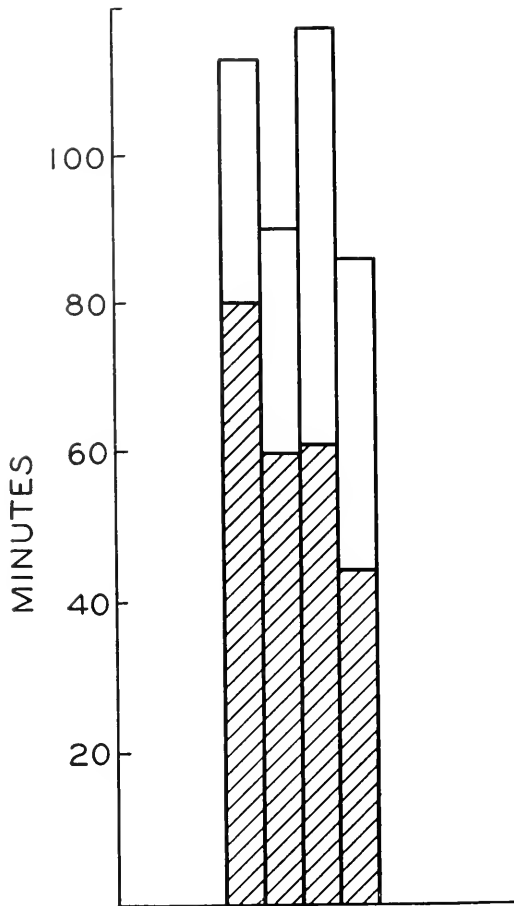


FIG. 3.—Effect of carbon dioxide accumulation on oxygen consumption in closed vessels. Time necessary for a rat enclosed in a vessel to diminish the partial pressure of oxygen to one-third of normal; when carbon dioxide is eliminated (black portion), and when it is allowed to accumulate (whole bar). Each bar represents an experiment on a different animal. Capacity of the vessel: 1 liter; ambient temperature: 20° C.; weight of animals: 150–180 grams.

fallen to dangerous values. In addition to these experimental results, other data from the literature show that carbon dioxide has a specific beneficial effect on the resistance to oxygen lack. There are also indications that it has a special protective effect against disorders elicited by the fall of body temperature, but this has not yet been proven.

The closed container technique uses both hypoxia and hypercapnia as agents to induce hypothermia. The convenience of the technique consists in the fact that both are produced by the animal itself and that it is only necessary to choose the right size of the vessel and the ambient temperature in order to achieve the wanted level of hypothermia without the danger of asphyxiation and other harmful effects.

The modifications of the method. Although less convenient, it is possible, as already mentioned, to achieve cooling by hypoxia alone (by decreasing gradually

the total barometric pressure with a vacuum pump, or by absorbing the expired carbon dioxide in a container of appropriate size). It is also possible to use hypercapnia alone for inducing hypothermia (by enclosing the animal in a vessel filled with pure oxygen), but we have found that under these conditions and by the time the body temperature has fallen below 20° C. the accumulation of carbon dioxide reaches values which are very close to the lethal concentrations. Many of our rats cooled by this technique succumbed even when returned to normal air. It is to be noted that carbon dioxide is much more lethal for the animal in hypothermia (Gjaja and Markovic, 1953). The closed container technique, with ordinary air and with no carbon dioxide removal, remains the simplest and safest method in-so-far as the rat is concerned.

Although the closed vessel remains our technique of preference for rats and other small laboratory animals, it is less convenient for such animals as the monkey and the dog where the size and surface ratio becomes an important limiting factor. The larger the animal, the slower is the rate of cooling and, consequently, the greater the danger of asphyxiation occurring before low temperatures can be reached.

Despite the size factor, we used the closed vessel technique in our experiments with monkeys as shown in the film. The animal is enclosed in a large perspex box and immediately anesthetized by introducing pure carbon dioxide into the box. The box is then placed in a refrigerator and refilled with nitrogen containing 7 per cent oxygen. From then on, the expired carbon dioxide is allowed to accumulate.

It is obvious, however, that by such a technique (cooling in air at 0° C.) the rate of cooling is always much slower than when cooling is achieved by immersion in icy water. It may be mentioned that carbon dioxide can be very useful for the suppression of shivering during cooling by immersion. We are now cooling dogs down to 15° C. by immersion, using only carbon dioxide and intravenous alcohol for narcosis and suppression of shivering.

Final cooling to 0° C., and supercooling. Once the body temperature is lowered to 15–20° C., the animal is removed from the vessel and further cooling is achieved by covering the animal with crushed ice. The animal is cooled to 0° C., and then supercooled by immersion in a propylene glycol or glycerol bath kept at –6° C. No artificial respiration or circulation is applied in the last stage of cooling because respiratory and cardiac arrest have already occurred (see fig. 4). To date, the lowest temperature from which we have resuscitated animals has been about –5° C.

RESUSCITATION BY LOCAL HEATING OF THE CARDIAC AREA

In 1951 we reported a technique for resuscitation of animals cooled to body temperatures around 0° (Andjus, 1951). Applying this technique, we have succeeded in reanimating adult non-hibernating mammals cooled to body temperatures reaching even a few degrees below the freezing point (Andjus, 1955).

Our experiments proved that deeply cooled animals with a cardiac arrest lasting up to almost two hours and body temperatures reaching about –5° C. can be resuscitated by localized external application of heat to the cardiac area. This strategy re-established the adequate circulation of blood ahead of the tissue requirements for

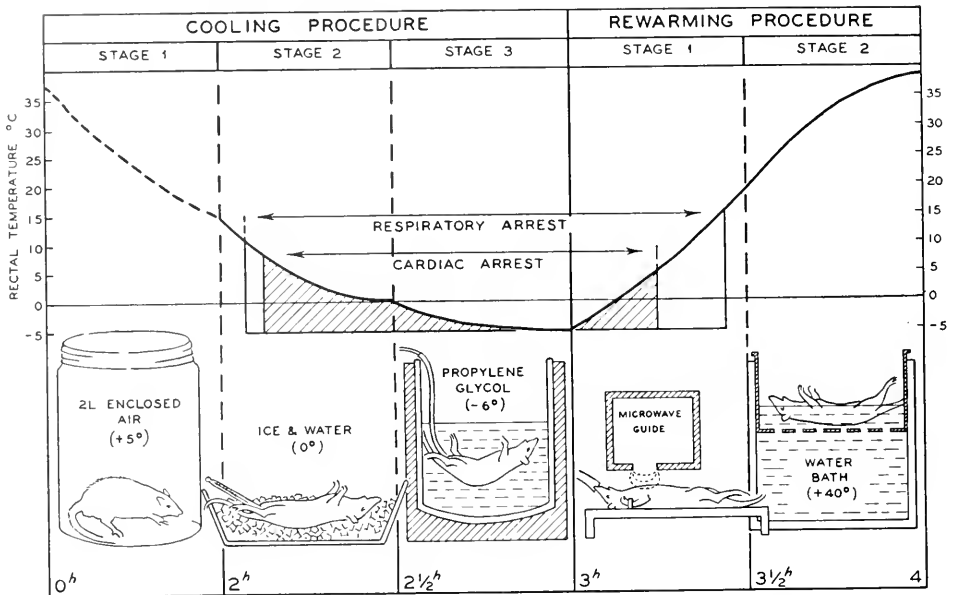


FIG. 4.—Illustration of the cooling and rewarming stages and procedures.

oxygen. Different heating devices were tested but the use of diathermy shown in the film proved to be the most successful (fig. 5). Maximal rate of recovery in rats (80–100 per cent of long-term survivors) was achieved by the use of microwave diathermy whose penetration properties allow localization to a small area (Andjus and Lovelock, 1955). For monkeys, however, a more conventional diathermy was used (30 Mc.) because of the need for deeper penetration. Our greatest difficulty with monkeys was the danger of superficial burns. Blocks of ice placed between the diathermy electrodes and the body surface diminished that danger.

THE PHYSIOLOGY OF INDUCED HYPOTHERMIA

The physiology of hypothermia varies markedly with the technique, especially if pharmacological agents are used. For example, at a body temperature around 30° C., the metabolic rate can range from readings higher than normal BMR to values many times lower, depending solely on the use of anesthetics.

Also, the physiological features of hypothermia at one level change appreciably with time. For example, at a certain level of hypothermia the metabolic rate can be significantly higher than the BMR during the first hours and significantly lower for many hours afterward. The blood sugar level can change from hyperglycemic to hypoglycemic values.

The hypothermic states. It is well known that the fall of body temperature does not influence all physiological functions in the same manner. The general classification of hypothermia stages which we are using is based on experiments with unanesthetized animals (fig. 6).

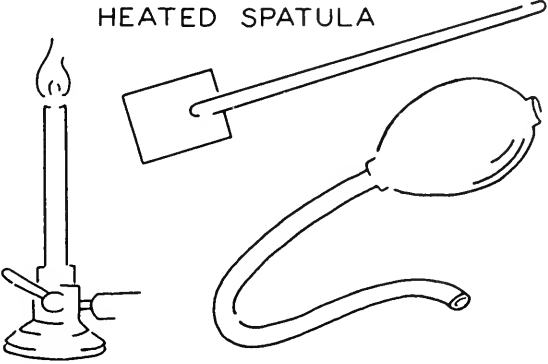
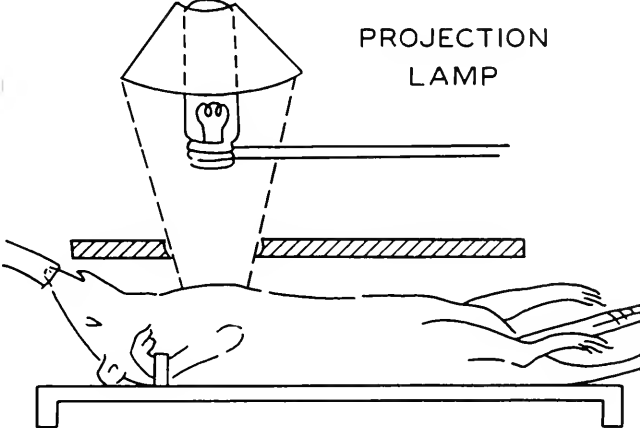
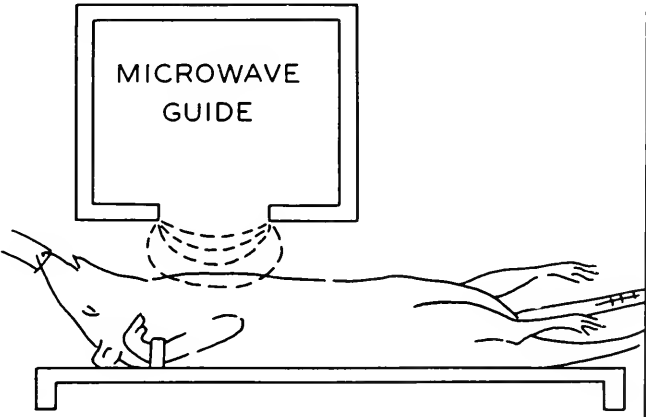
TECHNIQUE OF REANIMATION	% SURVIVORS
<p data-bbox="300 239 577 265">HEATED SPATULA</p> 	20%
<p data-bbox="505 661 695 722">PROJECTION LAMP</p> 	68%
<p data-bbox="258 1138 443 1199">MICROWAVE GUIDE</p> 	100%

FIG. 5.—Different techniques of local heating during reanimation from suspended animation at 0° C. of body temperature and their efficiency in terms of the number of survivors (maximal per cent of long-term survivors from 25 animals). From above downward: application of a heated spatula to the chest; artificial respiration by a bellows applied to the nostrils (Andjus, 1951; Andjus and Smith, 1955); heating by a projection lamp (Andjus and Smith, 1955); heating by microwave diathermy (Andjus and Lovelock, 1955; Andjus, 1955).

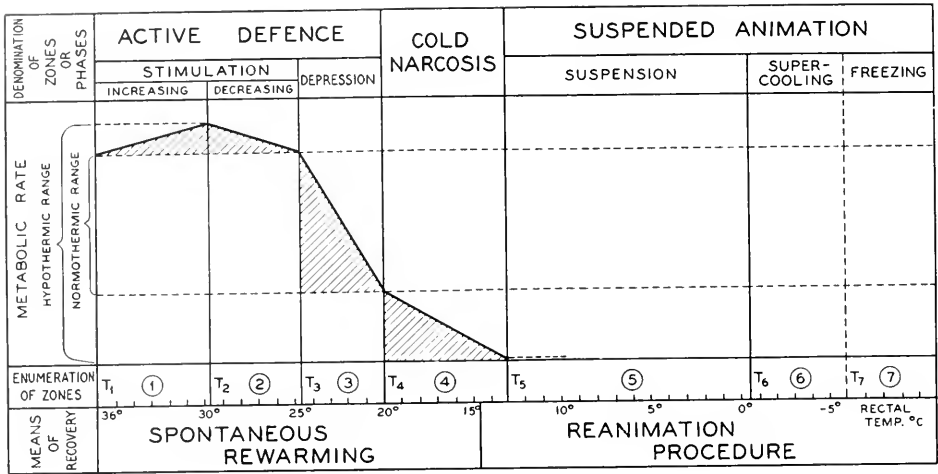


FIG. 6.—A schematic classification of hypothermia. T₁ = normal body temperature; T₂ = inversion temperature (inversion of temperature coefficient, peak of metabolism); T₃ = critical temperature (onset of the fall of metabolic rate below the prehypothermic level); T₄ = limit-temperature (starting the depression below the BMR level); T₅ = suspension temperature (respiratory, then cardiac arrest), T₆ = freezing point; T₇ = supercooling limit. Data based upon the rat.

The clinician should be aware of the physiological characteristics of the different states in order to choose the most suitable level of hypothermia. If a substantial decrease of the metabolic rate is desired, fig. 6 shows that this can be obtained in the animal only in the range of cold narcosis or below. The same is true if "physical anesthesia," induced by cold only, is wanted. Should a long reversible cardiac arrest be desired, the range of suspension would be best.

The experimental physiologist and biochemist can find many interesting problems associated with hypothermia. For example, in the range of active defence, at temperatures between 20 and 24° C., the glomerular filtration is still present in the rat's kidney, while the processes of reabsorption of some ions (sodium) are inhibited (Andjus, data presented to this Conference). The fixation of iodine and the synthesis of iodinated organic compounds in the thyroid gland, though greatly slowed down, are still present even in an animal maintained at a body temperature of 15 to 18° C. (Andjus *et al.*, 1954).

Maintained hypothermia. Comparing the time limits compatible with survival in the lowest ranges of hypothermia (from 25° C. downward), we found that the maximal survival time in rats decreases from about 50 hours at 23–25° C. of body temperature to two hours at 0° C. It is obvious, moreover, that the cause of death cannot be the same in the animal maintained at 0° C. of body temperature, with arrested circulation and respiration, as in the animal kept at 23° C., with a relatively high metabolic rate and even the capability of locomotion. If survival at 0° C. (determined by resuscitation) is compared to survival in other ranges of hypothermia with artificially arrested circulation, then our data speak in favor of the lowest range of temperatures.

The range of active defence. Two changes occur in animals maintained at 23–25° C.

(1) During the first phase (lasting about 20 hours), the animal actively attempts to rewarm. It is capable of regaining spontaneously its normal body temperature if placed in air and at a temperature somewhat lower than its body temperature. The longer, however, the period of continuous hypothermia prior to the beginning of rewarming, the longer it takes for spontaneous recovery (five and eight hours if rewarming is initiated after 8 and 20 hours of hypothermia, respectively).

(2) During the second phase (20–30 additional hours), the animal is deprived of the capability of spontaneous rewarming. If rewarmed artificially by external heat during the first few hours of this poikilothermic phase, the animal recovers completely. Later still, it does not respond to passive external rewarming. It dies during such rewarming, usually before normal body temperature is reached. In other words, long before the rat dies of continuous hypothermia, the possibility of recovery by simple rewarming is lost.

Figure 7 illustrates the above phenomena in terms of body temperature. Unanesthetized rats were maintained in hypothermia by immersion up to the neck in water of the same temperature as the cooled body. During the first 21 hours during which the animal is capable of spontaneous rewarming in air, the body temperature levels out at about two degrees above the temperature of the surrounding water after an initial four hours' period of maximal rise, corresponding to a peak of oxygen consumption. The second phase, during which spontaneous rewarming in air is impossible, is initiated by a narrowing of the temperature difference to about one-half a degree Centigrade. Figure 8 shows that during the first five hours of immer-

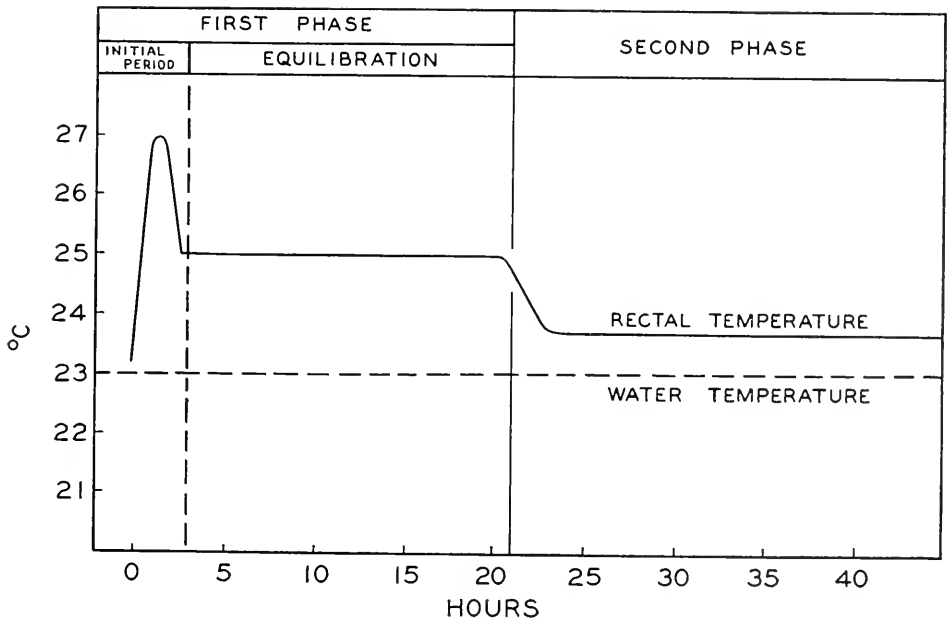


FIG. 7.—Changes in course of maintained hypothermia. Rats cooled by the closed vessel technique to a given body temperature and then immersed in water of the same temperature.

sion, the BMR undergoes tremendous changes, passing from values double normal to values below normal.

At the moment of death, liver glycogen stores are greatly reduced and severe hypoglycemia is present. A 24 hours' starvation prior to cooling shortens appreciably the tolerance to the duration of hypothermia.

The range of cold narcosis. Rats maintained in cold narcosis at about 15°C . differ from those kept in the range of active defence by a much shorter survival time (10–12 hours). Also, the changes in blood sugar and liver glycogen are less significant at the time of death. Hypoglycemia precedes death in the range of active defence, but hyperglycemia is usually still present in death at 15°C .

The range of suspended animation. The changes taking place with time in animals kept in suspended animation at 0°C . of body temperature are more difficult to detect. Death can be ascertained only by the failure of reanimation. The rate of complete recovery decreases as the duration of suspended animation is prolonged, and the rate of secondary deaths, occurring after partial recovery, increases until any recovery becomes impossible (fig. 9).

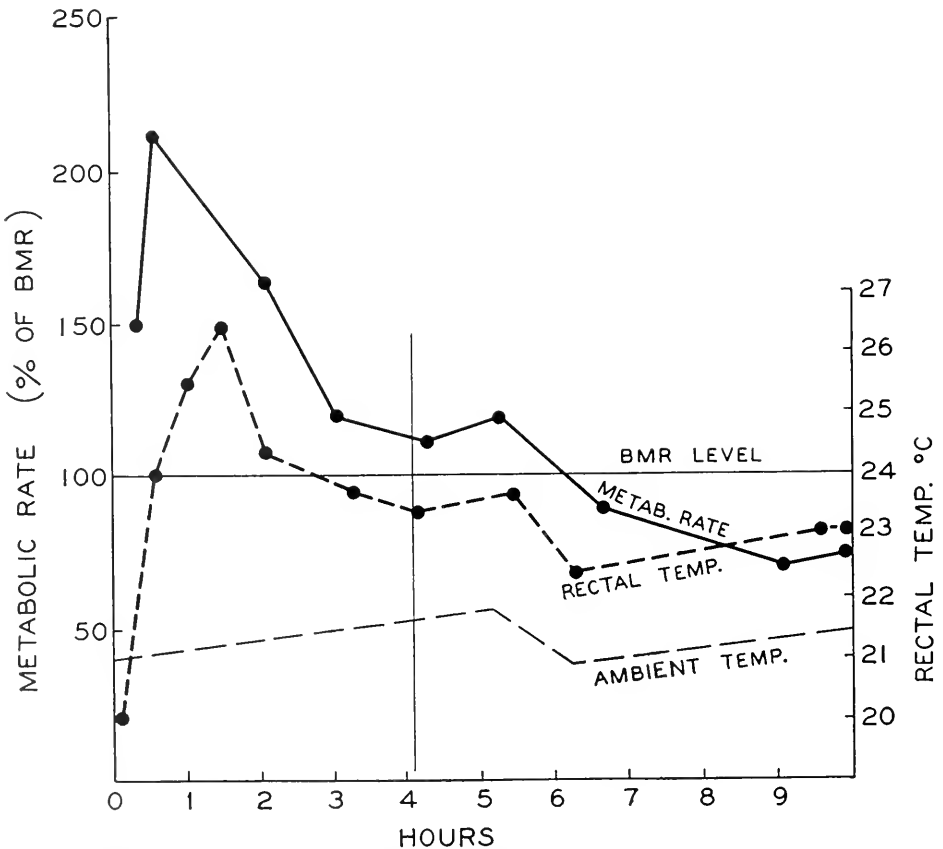


FIG. 8.—Metabolic rate and body temperature during the first 10 hours of immersion. A vertical line marks the moment of equilibration between body and water temperature.

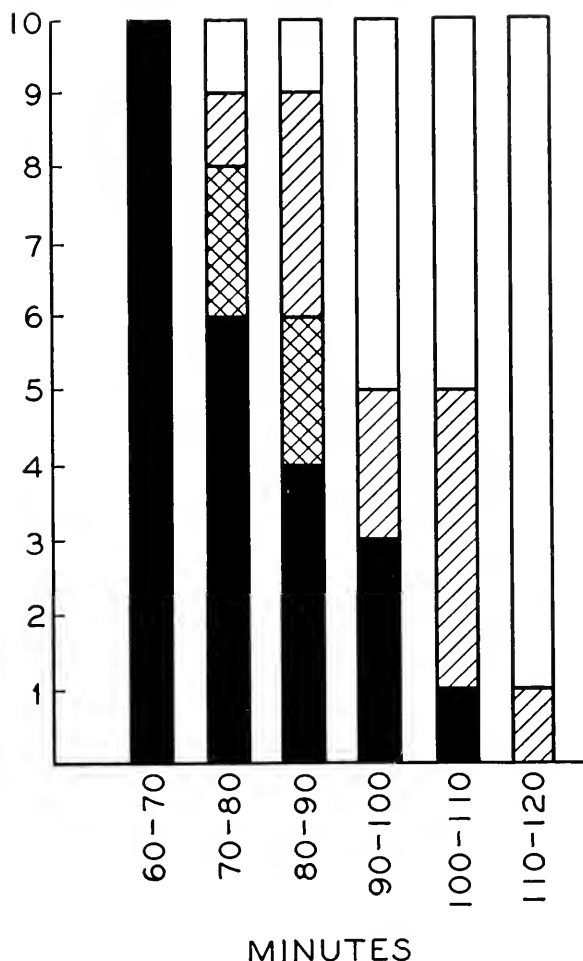


FIG. 9.—Effect of the duration of suspended animation in rats maintained at 0°C . of body temperature on the rate of recovery. Number of rats: whole bar—total number of individuals in an experimental group; black areas—long-term survivors; double shaded areas—dead within 10 days; simple shaded areas—dead within 24 hours; white areas—failed to revive. (Data from Andjus, 1955.)

After effects. The physiological changes recorded after normal body temperature is re-established differ greatly according to the level of hypothermia reached. Animals were not maintained longer than two hours at a given level of hypothermia in experiments referred to here. Rats cooled not lower than 15°C . re-establish their normal metabolic rate, thermoregulation, and blood constitution soon after normal body temperature is reached again. Blood sugar concentration, for instance, reaches normal values by the time the normal body temperature is regained, regardless of whether the latter is reached in 30 minutes or in two hours. Psychological tests showed no impairment of learning capacity and retention (Andjus *et al.*, 1955). It is important, however, to note that some changes which were not elicited during cooling, appear on rewarming. For example, the marked sodium retention by

the kidney takes place only in the last phase of rewarming (Andjus and Morel, 1952).

Rats reanimated from the state of suspended animation after cooling to zero or below have a much longer period of convalescence. The BMR is lower than normal and the RQ is abnormally high. Heat regulation is greatly impaired so that the behavior is poikilothermic during the first hours, even days, requiring warm surroundings. There is a temporary fall of water and food intake and a significant weight loss. There is also a temporary impairment of fertility (Andjus and Smith, 1955). Psychological tests showed that although retention is not significantly influenced, the learning capacity (problem-solving performance) seems to be significantly, although temporarily, impaired. One rat rewarmed after cooling to a body temperature of -3°C ., had to be fed artificially for days before its capability of standing and locomotion was re-established and its growth resumed.

Effect of repeated cooling. Rats can tolerate repeated cooling to zero, as frequently as ten times in the course of 43 days (Andjus, 1955). Repeated cooling, however, has its specific physiological consequences. Repeated cooling to 15°C ., for instance, increases the hyperglycemic reaction. Cooling to this level can be done as often as every second day for five weeks (Popovic, 1952). Of great interest are the adaptive changes elicited by repeated cooling to 0°C ., which result in significantly improved recovery rates, including more immediate re-establishment of thermoregulation, less weight loss, and a rapid resumption of growth. There is also an indication that repeated exposures lengthen the survival times after suspended animation (Andjus, 1955). The physiological mechanisms of these improvements remain unknown, although such changes as an increased adrenal weight have been recorded.

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DISCUSSION

Dr. F. John Lewis: My colleague, Dr. Niazi, has been able to cool several warm blooded animals to the levels reached by Dr. Andjus in rats. We lower the body temperature of mice, dogs, and monkeys to about 0° C. We have cooled rats to as low as minus 4° C. with survival, but unlike Dr. Smith's hamsters which are super-cooled, we have never produced ice in them. The technique is different from that employed by Andjus and Smith but many of the physiological changes are probably the same. It has pleased us to learn that it is easier to cool monkeys to these low temperature levels than it has been to cool either rats or dogs. It is about as easy to cool mice as it is monkeys.

The technique involves the use of artificial respiration throughout. In the mice, rats, and monkeys 5 per cent carbon dioxide in oxygen is given during cooling until cardiac standstill occurs. Standstill seems to be important in surviving the very lowest levels of hypothermia. In the dogs, it has been necessary to use a different technique in order to get a fairly high percentage of standstill; for the dogs the carbon dioxide is discontinued and oxygen is given at temperatures below 20° C.

The movie shows the incubation of the monkey after anesthetic induction with pentothal. We have used larger doses of pentothal than are usually used for lesser degrees of cooling. The electrocardiograph leads are attached and the monkey is cooled in blankets containing coils.

This shows the function of the artificial respirator. The respiratory rate was about 10 to 12 per minute. As the temperature went down, the cardiac rate slowed. The electrocardiograph shows the changes that Dr. Hegnauer demonstrated so well earlier.

You can see now that the rate is much slower. An hour and forty minutes after cooling the heart stopped at about 14° C. When the heart doesn't beat for five minutes we discontinue the respiration. In this case the animal's heart was in standstill with the respiration stopped for 56 minutes.

In rewarming we use hot water and warm only the chest to begin with. If you warm the entire body you are apt to get neurological damage. The lowest temperature reached in this animal was 9.5° C. Monkeys have been cooled to 4 or 5° C. We have done only five but four of them survived. After this animal was rewarmed, the heartbeat returned to about 180 per minute, the normal rate. The animal, ten minutes after his temperature had returned to 36° C., still looked a little groggy.

Dr. John W. Severinghaus: What was the oxygen tension in the jar when the animals were removed?

Dr. R. K. Andjus: The CO₂ concentration was 16 per cent, and that of O₂ was low, about 3 per cent.

Dr. W. Parkins: How long did it take to freeze these animals? Did you keep them frozen for more than an hour?

Dr. Audrey U. Smith: Freezing usually started about 10 minutes after immersion in fluid at -5° C. One hour of freezing at -5° C. was just about the limit for reviving hamsters by the simple method shown. When we froze them for 75 minutes they recovered apparently completely, but they usually died within a few hours. The commonest cause of death was gastric hemorrhage, so far as we could tell from autopsies. Animals frozen for as long as 96 minutes have recovered heartbeat and breathing but they usually died without recovering posture and consciousness. Animals frozen for 180 minutes have recovered heartbeats only.

Dr. Andjus: As to the rat, the longest period below zero in the rat was 40 minutes with subsequent recovery. The lowest temperature reached was -3.3° C. in the rectum and -5.7° C. under the skin.

Dr. A. L. Hopkins: I would like to ask if you had the formation of ice in the tissues of these animals or if you had vitrification.

Dr. Smith: There is no question about it. The animals either froze or became supercooled. In the frozen animals masses of ice were present in the tissues. Some of the animals were cut in half instead of being resuscitated. Ice crystals could be picked out of the body wall, the peritoneal cavity, the hollow of viscera and solid organs. There was no doubt about the presence of ice. We were cooling the animals slowly; the physical conditions were not those under which vitrification could have been expected.

Dr. Jacob Fine: How do you explain the effect of the time factor in that case?

Dr. Smith: During immersion of a body in a sub-zero bath, freezing occurs gradually. The ice front progresses inwards from the surface to the interior of the animal. The longer the duration of freezing, the higher the proportion of water frozen in any situation. At the end of an hour of freezing, 90 per cent of the water in the skin may be converted into ice, and as much as 50 per cent of the total body water may be frozen. In some animals as much as 62 per cent of the water in the brain has become frozen. Meanwhile the concentration of electrolytes in the tissue fluid surrounding the individual cells is increasing and will eventually reach a level which damages the various cells and tissues. Unless the whole animal can be permeated with at least 10 per cent of glycerol we do not think we will be able to freeze 100 per cent of the body water and revive the hamster afterwards.

Dr. R. W. Brauer: Dr. Smith was kind enough to give us the operating instructions for their cooling procedures. Being curious about the oxygen in those, we have repeated some of the experiments since, using 100 per cent oxygen instead of air in the jar. Those animals go out precisely as rapidly as the ones in the air-filled jars and, furthermore, if you control doing the same animal repeatedly, on alternate days with oxygen and with air, the temperature lowering proceeds equally well under both conditions.

Dr. F. D. Moore: What happens if you do not build up the CO_2 ?

Dr. Andjus: If an animal is enclosed in the jar and the CO_2 is absorbed, then in the same period of time the animal will not cool sufficiently and will die. Conversely, if the CO_2 is allowed to accumulate, the animal will cool below 20° and will survive that confinement. The simplest and most effective way is to combine the two; oxygen lack and CO_2 excess.

Dr. Dripps: Most effective in what way—rapidity, survival?

Dr. Andjus: If you enclose a rat in a jar of a certain capacity and allow the expired CO_2 to accumulate, the animal cools down below 20° of body temperature and survives for two hours in the closed vessel. If the expired CO_2 is being absorbed all the time, you find that the animal, all other conditions being identical, does not cool to the same extent and dies after a shorter period of confinement.

Dr. Jean Henley: Do you get fibrillation of the heart?

Dr. Andjus: I don't think so; that is, we do not have any evidence of fibrillation.

Dr. Smith: I think that occasionally hearts may fibrillate during rewarming. In some of the rats which failed to revive fully, the heart was fibrillating at autopsy. Sometimes when I had failed to revive a frozen hamster I found that the heart was fibrillating when I opened the chest. We didn't record fibrillation in any of the electrocardiograms.

PART II

CARDIOVASCULAR FUNCTIONS IN DEEP HYPOTHERMIA

HENRY E. D'AMATO

In the preparation of this paper it was not the intention of the author to review exhaustively all published material pertaining to the general subject. Such treatment would require a monograph. Rather, it was intended that this paper should constitute a brief demonstration, by means of published data, of the observed major alterations in the dynamic functions of the mammalian heart and peripheral vessels. Unless otherwise stated, the paper will ignore the occurrence and consequences of all cardiac arrhythmias. Also, unless otherwise stated, all departures from normal which are observed in deep hypothermia are completely reversible upon rewarming.

Heart rate and arterial pressure. The profound bradycardia which is characteristic of the dog in deep hypothermia is shown in figure 1. The graph was drawn from the tabulated data of Hook and Stormont.¹ The figure illustrates the marked, progressive slowing of the heart rate from 160 beats per minute at a rectal temperature of 38° C., to about 20 beats per minute at 18° C.

Hegnauer, Schriber and Haterius² recorded the pulse rate of dogs at shorter intervals throughout the cooling process and obtained the average curve shown in figure 2. At corresponding temperatures the heart rates of figures 1 and 2 are almost identical.

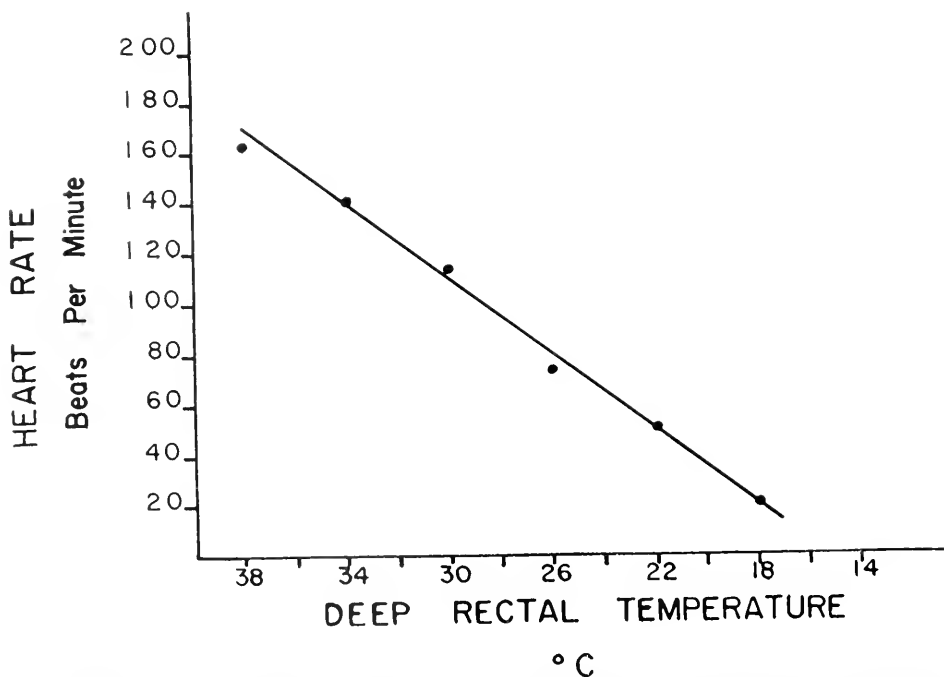


FIG. 1.—Heart rate in hypothermia. (From Hook and Stormont, *Am. J. Physiol.* 133: 334, 1941.)

Figure 2 demonstrates the occurrence of cardioacceleratory reflexes in the early stages of hypothermia. The first point on the curve represents the pulse rate prior to immersion into an iced bath. Upon immersion, a marked increase in rate occurs, apparently referable to reflex sympathetic discharge in response to the noxious stimulus. At subsequent points on the curve the observed pulse rate results from the modification of the control rate by two opposing factors, reflex stimulation and direct depression by the cold. As we shall see, this situation obtains in several other cardiodynamic functions.

The greater part of this curve represents the average of nineteen experiments. The term "pulse rate" includes both sinus beats and effective beats of ectopic origin. In three of the nineteen dogs, the hearts were observed to beat down to a heart temperature of 14° C. In all three dogs, a slight increase in pulse rate occurred between 16° C. and 14° C., perhaps due to an increase in ectopic activity just before terminus.

In the same series of dogs, pressure in the femoral artery was simultaneously measured. Apparently systemic arterial pressure is to some degree independent of pulse rate down to blood temperatures of 24° C. to 23° C. However, at this point it becomes completely dependent on pulse rate. The exact cause of the abrupt change in the curve is questionable. It could represent vasomotor paralysis, or could result from complete cessation of shivering which invariably occurred at or before these temperatures. This curve is in essential agreement with those observed by other workers.^{1, 3, 4, 5}

Nature of bradycardia. Bradycardia induced by hypothermia differs from that induced at normal temperature by stimulation of the vagus nerve.² Typical tracings of left intraventricular pressure in the dog are shown in figure 3. One may, for the sake of convenience, consider systole to be represented by that part of the pressure

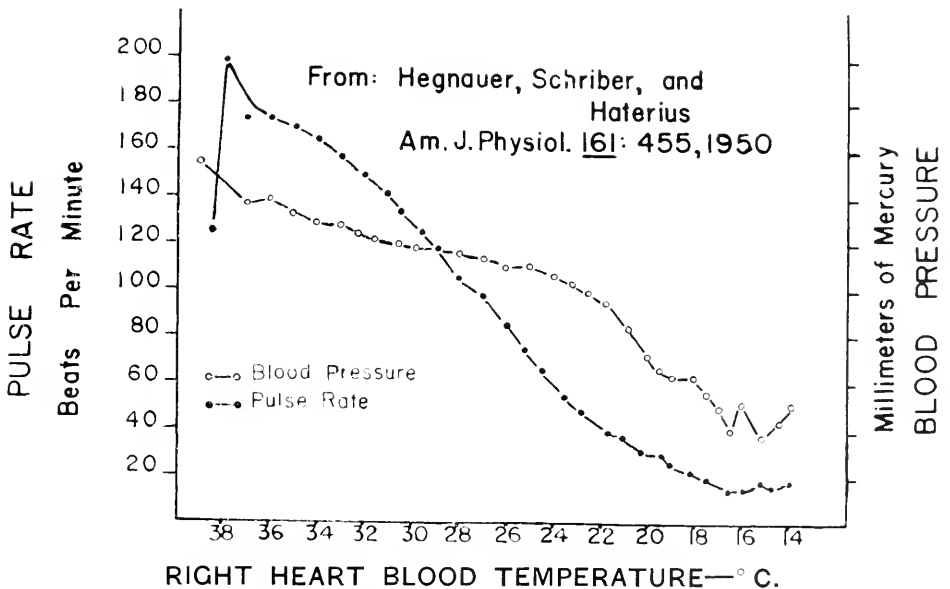


FIG. 2.—Blood pressure and pulse rate in hypothermia.

CYCLIC RELATIONS OF TRACINGS OF LEFT VENTRICULAR PRESSURE CURVES

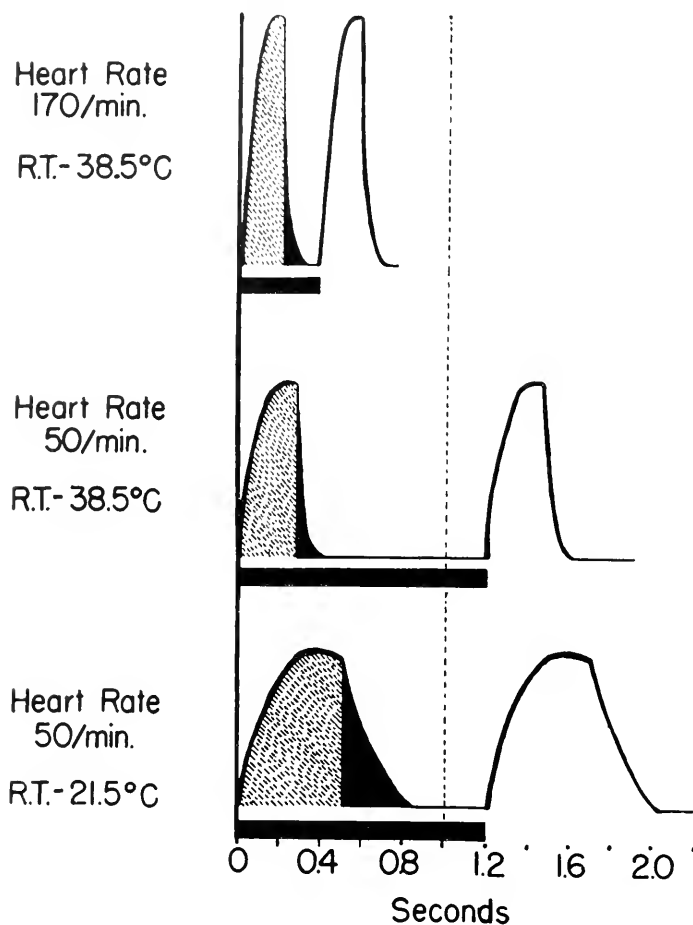


FIG. 3.—Duration of systole, isometric relaxation and total cardiac cycle at normal heart temperature and at 23° C.

contour from the point at which the curve leaves the baseline to the point at which the abrupt fall toward baseline begins. One may further consider isometric relaxation to endure from this abrupt fall in the contour to the point at which the curve once again reaches the baseline. The two together constitute the "activity phase" of the cardiac cycle. The remainder of the cycle represents the "resting phase."

It will be noted that with a heart rate of 170 beats per minute at 37° C., systole persists for a little more than fifty per cent of the total cycle. When the same heart at the same temperature is slowed to fifty beats per minute by stimulation of the vagus nerve, the total cardiac cycle length increases by over 300 per cent but the duration of systole increases only 50 per cent. Isometric relaxation, meanwhile, does not change appreciably. When the heart at normal temperature is slowed, the length of the so-called resting period profits.

On the other hand, when the heart is cooled to 23°C ., to a heart rate of fifty beats per minute, the total cycle also increases by 300 per cent, but the durations of both systole and isometric relaxation increase by 250 per cent. In other words, when the heart is slowed by hypothermia the resting period undergoes no great relative increase in duration.

This phenomenon is illustrated in figure 4. As heart temperature decreases, the absolute time of systole and the absolute time of isometric relaxation increase. These curves represent the averages obtained from a large series of dogs. The data are from pressure contours which are "normal" for the given temperature. Those from irregular cycles, ectopic beats, etc., are not included.

In figure 5 the same data are expressed as the per cent of the total cycle represented by the activity phase in relation to the pulse rate. At normal temperature, there is a progressive decline in relative length of the activity phase throughout the entire range of pulse rate. When the heart is slowed by cooling there is no great change in the relative length of activity phase down to pulse rates of fifty to sixty beats per minute, which occur at 23°C .. With further cooling and further slowing of the pulse rate, there is an abrupt decrease in the relative length of activity phase. At a pulse rate of twenty beats per minute, at a temperature of about 18°C ., the relative length of the activity phase of the hypothermic heart is still far greater than that of the normothermic heart beating at a similar rate. Berne⁶ has since corroborated these results.

Coronary circulation. In view of these findings concerning the duration of systole and isometric relaxation in hypothermia, two questions arise with regard to the coronary circulation. Is the so-called "rest period" of sufficient length to insure

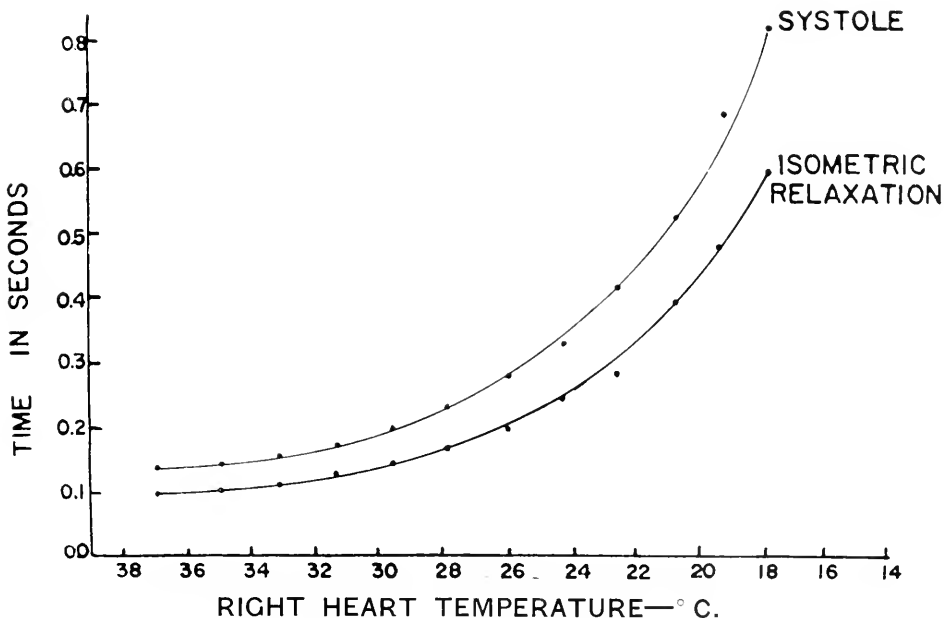


FIG. 4.—Duration of systole and isometric relaxation in the hypothermic dog.

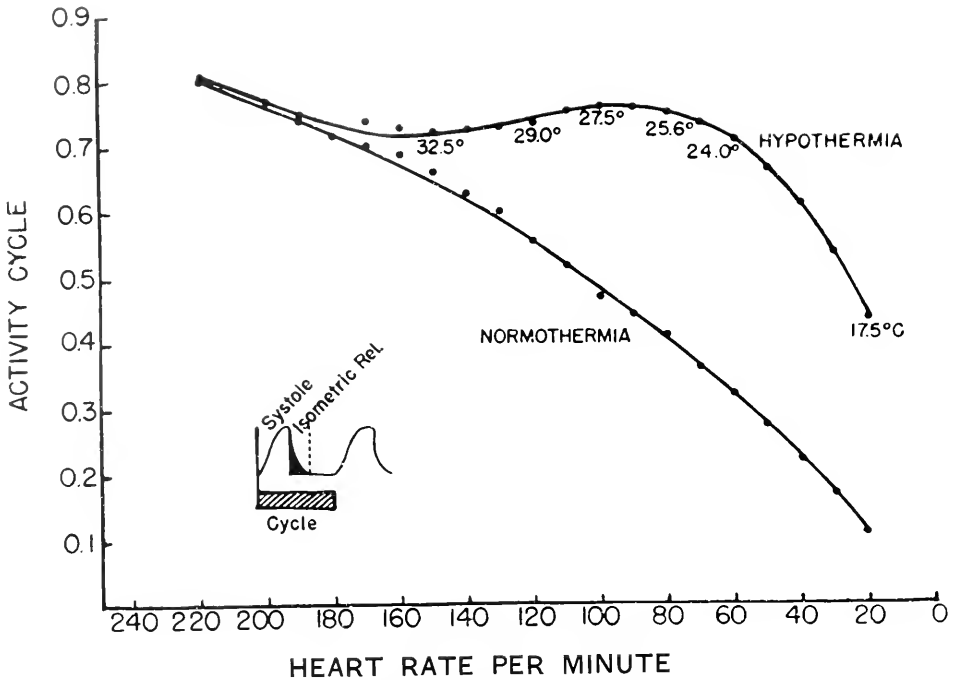


FIG. 5.—Relative length of activity phase with decreasing heart rate at normal temperature and in hypothermia.

adequate coronary blood flow? Can the heart extract from blood at approximately 20° C. an adequate volume of oxygen?

The adequacy of coronary blood flow has been demonstrated by Berne.³ He observed a difference in the slopes of the aortic pressure curve and the coronary blood flow curve. Aortic pressure decreased slightly in the early stages of hypothermia, then underwent a sharper decrease beginning in the vicinity of 28° C. In contrast, the coronary blood flow decreased sharply in the early stages, then at about 33° C. began a more gradual decline. Figure 6 illustrates the average coronary blood flow in thirteen hypothermic dogs. This graph depicts the relationship between coronary blood flow and the perfusion pressure behind the flow. In these experiments the perfusion pressure was that of the subclavian artery. The relatively sharp initial decrease in coronary blood flow is followed by a more gradual decrease beginning at a perfusion pressure of about 80 mm. Hg. At 19° C. the magnitude of coronary blood flow is approximately one-fourth that of control flow.

If this rather drastic reduction in coronary blood flow were the prime causative factor in some pathological state of cardiac function, then the experimental increase of coronary blood flow should relieve that pathological state. Berne controlled the volume of coronary blood flow by means of a pump perfusion system in parallel with his arterial perfusion system. The results of six experiments are summarized in table I. By raising the artificial pressure head above the normal for a given low temperature, Berne was able to increase coronary blood flow from 200 to 900 per

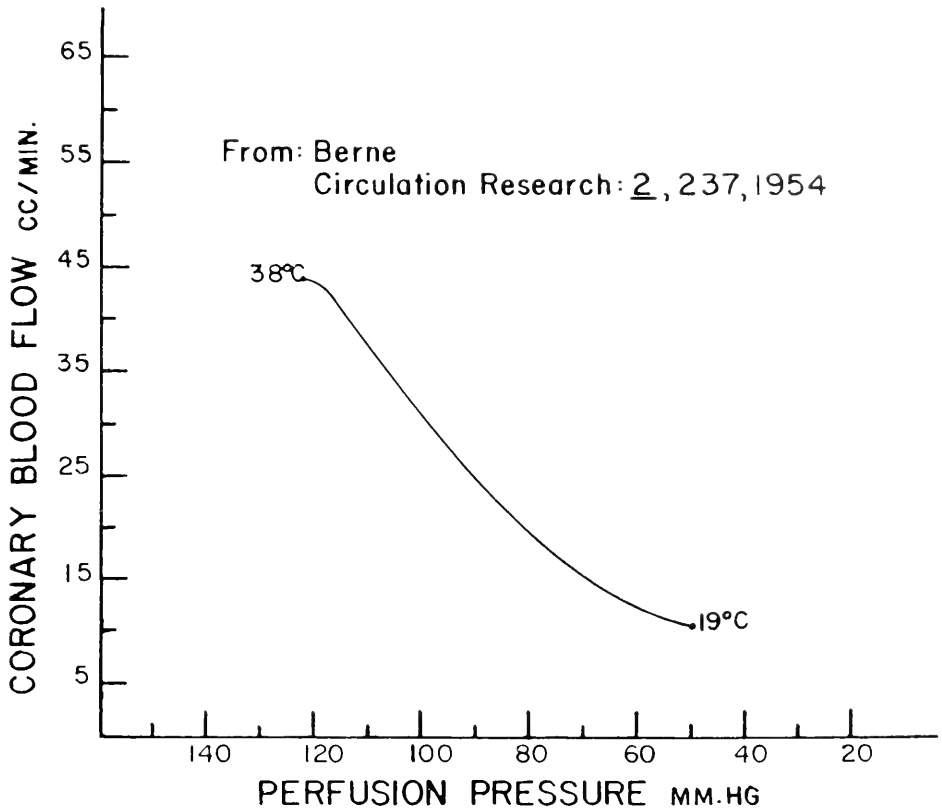


FIG. 6.—Coronary blood flow in the hypothermic dog.

TABLE I

EFFECT OF ELEVATION OF PERFUSION PRESSURE ON CORONARY BLOOD FLOW, AORTIC PRESSURE, AND HEART RATE IN THE HYPOTHERMIC DOG

Exp.	Right atrial temperature (° C.)	Coronary perfusion pressure (mm. Hg)	Coronary blood flow (cc./min.)	Aortic pressure (mm. Hg)	Heart rate (beats/min.)
7	{ 21.0	Without pump, 37	4.0	45/30	75
		With pump, 112	36.0	48/30	76
9	{ 21.0	Without pump, 62	18.5	80/51	48
		With pump, 87	38.5	81/51	46
13	{ 25.5	Without pump, 58	11.0	68/54	57
		With pump, 121	37.5	67/52	60
16	{ 19.0	Without pump, 46	14.0	67/40	31
		With pump, 117	74.5	68/41	32
18	{ 21.5	Without pump, 40	10.5	55/29	37
		With pump, 98	74.5	54/28	37
26	20.5	Without pump, 106	18.0	118/92	26
		With pump, 124	32.0	119/93	26

From: Berne, Circulation Research 2: 238, 1954.

cent. In no case did there occur an increase in aortic pressure or in heart rate. It therefore follows that at a heart temperature of 20° C. there is no functional cardiac abnormality directly attributable to diminished coronary flow.

A more important consideration in the assessment of the adequacy of the coronary circulation in hypothermia is the question of release of oxygen by cold blood to cold tissues. The displacement of the oxygen dissociation curve to the left by cold is well known. At a given partial pressure of oxygen, a greater per cent of oxygen saturation obtains in cold blood than in normal blood. This has been shown to be true *in vitro* by Brown and Hill⁷ and *in vivo* by Penrod.⁸ Does this phenomenon prevent cardiac tissue from getting an adequate supply of oxygen even though blood flow is abundant?

Penrod⁸ measured coronary arterio-venous oxygen differences in dogs at normal temperature and at blood temperatures of 20° C. His results are summarized in table II. The average coronary A-V oxygen difference in ten normal dogs was almost identical with that found in fifteen dogs at 20° C. A drastic reduction in the partial pressure of oxygen in coronary venous blood and presumably in cardiac tissue made this possible. It must be emphasized however that the coronary A-V oxygen difference in deep hypothermia, i.e., at 20° C., is the same as that which obtains at normal temperature. A similar situation exists in the dog at 17° C.⁹ Thus, per unit of blood, hypothermic cardiac tissue can extract as much oxygen as it can at normal temperature. Since the volume of coronary blood flow is adequate for the conditions, it must be concluded that an adequate volume of oxygen is taken up by cardiac tissue at this low temperature.

Cardiac output and work. In the early stages of hypothermia the volume of blood put out by the heart varies with the oxygen consumption of the animal, which in turn is directly related to the intensity of reflex shivering. When general anesthesia is light and shivering is violent, total oxygen consumption and cardiac output are increased two, three, or even four-fold. When shivering is moderate, cardiac output shows only a moderate increase over the non-shivering control state. When general anesthesia is sufficiently deep to eliminate shivering, cardiac output is diminished. Thus, we have another example of a function modified by two opposing factors, reflex stimulation and direct depression. At temperatures of 20° C. to 18° C., when shivering is absent, cardiac output is about 15 per cent of normal.

The observation of Hegnauer and D'Amato,⁹ summarized in table III, agree closely with those of other workers. Nine dogs at normal temperature showed an average cardiac output of 146 ml./kg./min. In eight of these dogs, at an average

TABLE II
CARDIAC OXYGENATION AT NORMAL TEMPERATURE AND IN HYPOTHERMIA

	Normal temp. (n = 10)	Blood temp. 20° C. (n = 15)
Coronary A-V O ₂ diff. (vol. %)	12.3	12.1
Coronary venous PO ₂ (mm. Hg)	19	7.6
Hematocrit (%)	41.9	50.1
Heart rate (per minute)	156	33.5
Arterial pressure (mm. Hg)	103	60

TABLE III
CARDIAC OUTPUT IN THE HYPOTHERMIC DOG

Temp.	No. of dogs	Art. O ₂ Vol. %	Ven. O ₂ Vol. %	A-V diff. Vol. %	Cardiac output ml./Kg./Min.
Norm.	9	20.1	14.1	6.0	145.5
17° C.	8	22.0	17.1	4.9	26.0
Expt. No. 12, 17° C.	1	23.0	3.9	19.1	3.2

temperature of 17° C., cardiac output was reduced to 18 per cent of control. The ninth dog, when cooled, presented a unique case. At 20° C. the pulse rate and blood pressure were 29 per minute and 68 mm. Hg., respectively. Then the heart suddenly stopped and the blood pressure fell to zero. Both heart rate and blood pressure recovered periodically until the rectal temperature reached 17° C., at which temperature these measurements were made. Undoubtedly, during these bouts of relative asystole a substantial oxygen debt was incurred. In the face of this oxygen debt a greater volume of oxygen was extracted from a unit of blood, hence the low venous oxygen content and the high A-V oxygen difference. During rewarming the A-V oxygen difference returned to normal (4.2 vol. per cent), indicating that the oxygen debt had been paid off. This demonstrates that in the presence of tissue hypoxia the required oxygen can be extracted from the blood in spite of the increased affinity of hemoglobin for oxygen at low temperatures. The failure of such increased extraction in the remaining eight hypothermic dogs indicates that the ordinary demands at the cold temperatures are met by other means.

In the same experiments cardiac work was computed at normal body temperature and at the cold temperature. The results are summarized in table IV. If we apply the simple formula $W = QR$, in which Q is the stroke volume and R is the mean arterial pressure, at normal temperature the work performed by the heart is 15.4 gram meters per stroke, or 2618 gram meters per minute. At the cold temperature these values become 7.6 gram meters per stroke, or 198 gram meters per minute. Thus the work performed by the heart *per minute* is reduced to seven to eight per cent of normal. The work performed by the heart *per stroke* is reduced only by 50 per cent.

Blood flow. The observations of coronary blood flow in hypothermia by Berne have been described in a previous section. Reductions in cerebral blood flow of similar magnitude (approximately 75 per cent of control) have been reported by Rosomoff and Holaday.¹⁰ In their experiments the diminution of cerebral blood flow was linear and directly proportional to an observed decrease in cerebral oxygen consumption. Cerebral arterio-venous oxygen difference remained unchanged, indicating an adequate supply of oxygen to the brain in hypothermia. Splanchnic blood flow has

TABLE IV
CARDIAC WORK IN THE HYPOTHERMIC DOG

Temp.	Blood pressure mm. Hg.	Stroke vol. ml.	Pulse rate per min.	Work per stroke gm. meters	Work per min. gm. meters
Norm.	126	9.0	170	15.4	2618
17° C. (16-18)	54	10.4	26	7.6	198

been observed by Hallett¹¹ to undergo a decrease to 22 per cent of control flow at 23° C. According to Page¹² effective renal plasma flow follows variations in cardiac output.

Thus it appears that the volume of blood flow to organs in hypothermia is closely related to the output of blood from the heart.

With regard to blood flow in the minute peripheral vessels, Bigelow⁵ examined microscopically the vessels of the conjunctiva in the hypothermic dog. He observed marked vascular stasis at the lower temperatures and complete cessation of flow in some arterioles and veins as large as 60 micra in diameter.

Summary. By way of summary, let us attempt to form some conclusions as to the state of the cardiovascular system in profound hypothermia. Consider the dog at a body temperature of 20° C. Its cardiac output is about 15 per cent of normal. Systemic arterial pressure is usually 60–70 mm.Hg. The work per minute performed by the heart is also about 15 per cent of normal. Stroke volume, on the other hand, is normal. It therefore appears that the marked reduction in these functions is referable to the extreme bradycardia which occurs at this low temperature.

At 20° C. the body tissues are apparently supplied with an adequate amount of oxygen, in view of the facts that there is no evidence of oxygen debt upon rewarming and that the tissues can extract more oxygen, when needed, by increasing the coefficient of utilization. Therefore, at this low temperature with its reduced oxygen requirements, the slowed heart rate in spite of all its physiological consequences is adequate, just as at 37° C. the control heart rate was adequate. In this somewhat restricted sense, the bradycardia at the low temperature is "normal" for that temperature.

The heart in deep hypothermia is usually described by the word "depressed." If by the word "depressed" we mean "slowed in rate of contraction," then obviously the heart in hypothermia is "depressed." Beyond this meaning, however, the word "depressed" is used inaccurately in this connection. The characteristics of a heart which has been depressed (in the classical sense of the word) by drugs include: (a) a more gradual contraction; (b) a lower amplitude of contraction; (c) a shorter duration of contraction; (d) a reduced stroke volume; and (e) an elevated initial intraventricular pressure.¹³ In hypothermia the last three characteristics are lacking and the second, viz., a lower amplitude of contraction, is not particularly dramatic.

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DISCUSSION

Dr. I. K. R. McMillan: I would like to start by saying that our group in England a year or two ago did a similar experiment on calcium [unpublished]. I am delighted to see our results agreed so closely. In fact, I would carry it a little further. By watching the EKG we could give calcium chloride and produce an ST elevation at temperatures where it does not normally appear (i.e., 28° C.). If we did it the other way around, and gave more potassium or alternately sodium acid phosphate and mopped up the free calcium ions at a temperature of 25° C., we could remove that elevation entirely and cool the dog several degrees further before it started to reappear. We have also been interested in the hemodynamics of the cold heart, and Dr. Case, Dr. Stainsby and I have been trying to quantitate the function of the cold ventricle along the lines of the work previously published by Dr. Sarnoff and his associates. In view of the diminution in cardiac output, coronary flow, and aortic pressure during hypothermia, it seemed desirable to examine the contractility of the myocardium in this state. The amount of work per stroke that the heart is able to deliver at any given filling pressure is a measure of its contractility, and is best expressed by ventricular function curves, as described by Sarnoff, Berghlund and Case.^{1, 2} For the determination of these curves, continuous, simultaneous recordings were made of cardiac output, left main coronary artery flow, aortic pressure, pulmonary artery pressure, and right and left atrial pressures. Myocardial work was increased by intermittent intravenous transfusion of blood until the work reached a maximum. Curves of filling pressure against stroke work were plotted for each ventricle at 37° C. and 28° C. All dogs were under morphine-chloralose-urethane anesthesia and positive pressure breathing. Blood was cooled by passage from the femoral artery to the femoral vein through a coil immersed in ice water.³

Values of the important hemodynamic variables as cooling progressed are shown in figure 1. The rise in filling pressures during cooling reported by other workers did not occur in these experiments.

Figure 2 shows a plot of left ventricular stroke work against mean left atrial pressure at 37° C. and 28° C. It will be noted that the stroke work generated per unit of filling pressure in the cold heart was about the same as that of the warm heart, suggesting that the contractility at the two temperatures was the same. However, present work in this laboratory indicates that the function curve is elevated progressively at normal temperatures as heart rate decreases. During these curves,

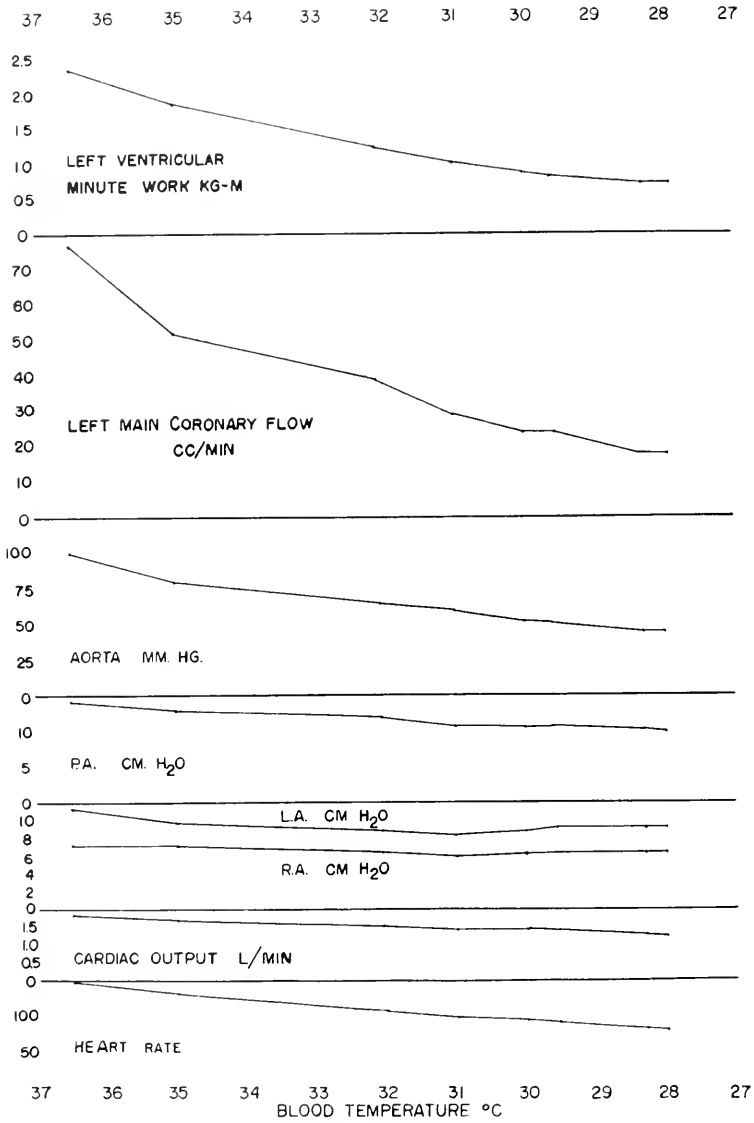


FIG. 1.—Hemodynamic data during cooling obtained from continuous, simultaneous recordings. All pressures are electrically determined means. Cardiac output measured by Potter Electroturbidometer. Coronary flow measured by rotameter. Dog weight 23.2 kg.

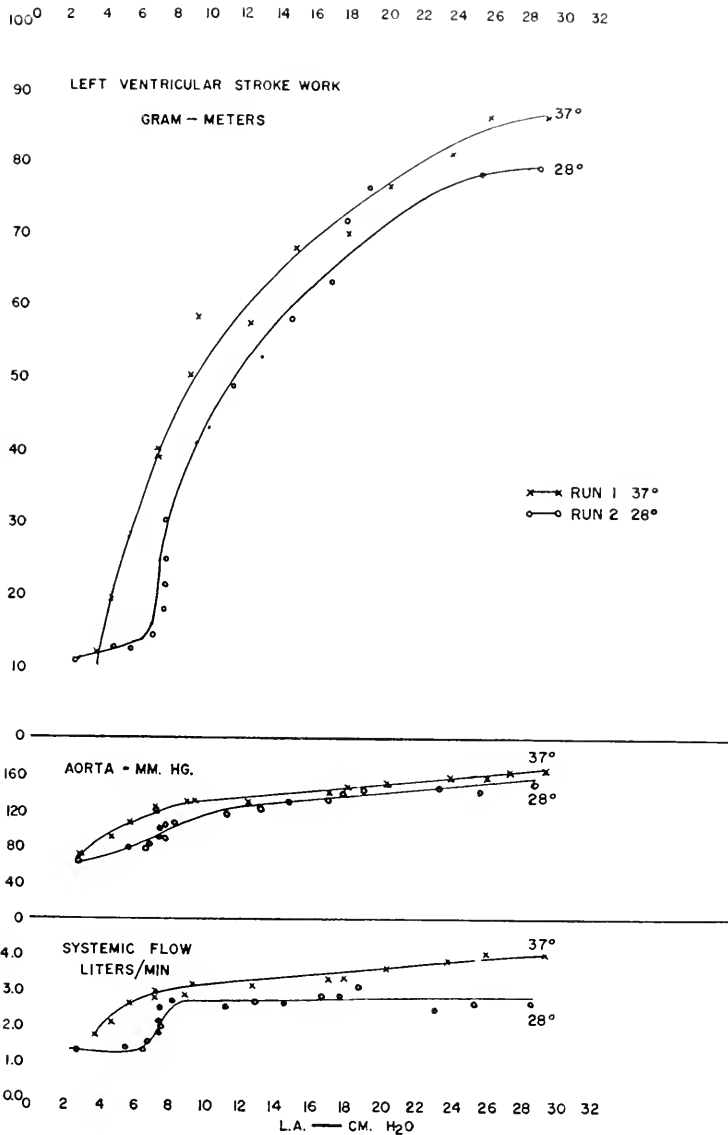


FIG. 2.—Relation of left ventricular stroke work, mean aortic pressure and systemic flow to mean left atrial pressure at 37° C. and 28° C.

heart rate at 37° varied from 128 to 85, while at 28° the rate was 100 to 61. These data suggest that there is a slight reduction in contractility of the myocardium at reduced temperatures, but that the reduction in heart rate in this state allows the heart to deliver a stroke work per unit of filling pressure equal to that of the normothermic state. In experiments in which a greater reduction in rate occurred in the cold heart, the ventricular function curve was higher at 28° than at 37° . Consideration of the output and pressure plots shows the very considerable increase in these values which could be achieved in the cold heart as well as in the heart at 37° .

An increased efficiency of the heart during hypothermia is suggested by figure 3 where a reduced amount of left main coronary flow per unit of left ventricular minute work is present at lower work loads. The range of aortic pressure was nearly identical for the three runs. A marked increase in efficiency of the normothermic animal occurs by simply reducing heart rate, and it is possible that this change in efficiency in hypothermia may be due solely to a reduced heart rate.

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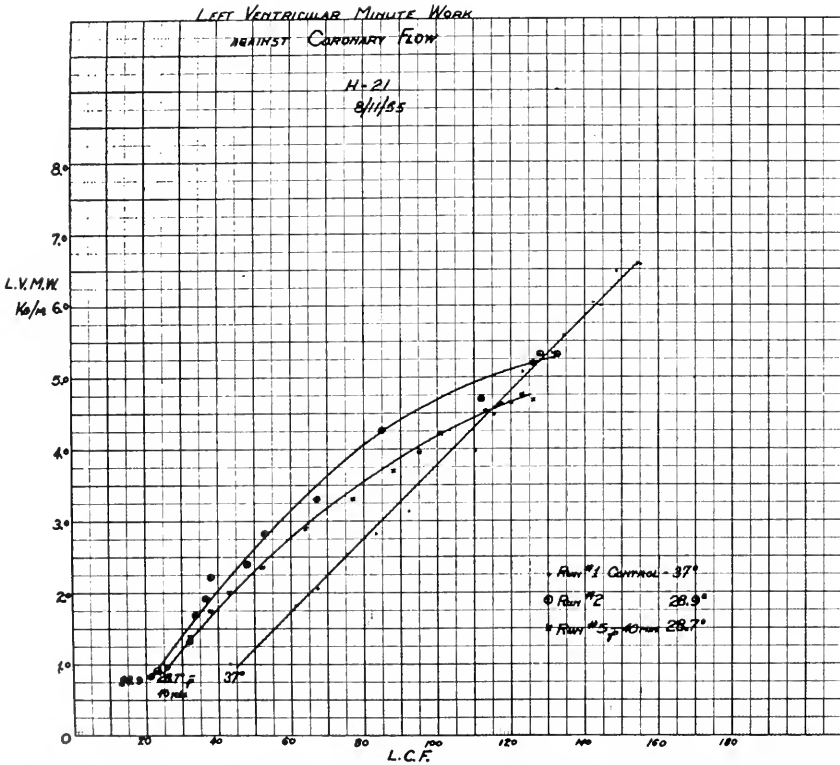


FIG. 3. Relation of left ventricular minute work (L.V.M.W.) in kilogram meters to left main coronary flow (L.C.F.) in cc/min

TABLE II

CARDIOVASCULAR AND RESPIRATORY DYNAMICS IN DOGS COOLED AFTER INDUCTION OF HEMORRHAGIC SHOCK

	Rectal temp. (° C.)	Arterial pressure (mm. Hg.)	Pulse rate (min.)	Cardiac output (ml.)	Arterial O ₂ (vol. %)	A-V O ₂ diff. (vol. %)	Pulmonary ventilation (L./min.)	O ₂ consumption (ml./min.)	Respiration rate (min.)
Prior to cooling									
or shock	38-39	136	150	2920	19.2	6.3	19.6	164.4	34
During shock	38-39	30	166	724	17.0	13.2	18.6	99.5	32
During hypothermia									
and shock	24	30	84	366	17.6	14.2	15.5	68.8	21
After transfusion									
and rise in body									
temperature	33.2	106	90	4752	19.1	4.2	22.7	193.4	21
Number of experiments									
for each value listed	—	(10)	(10)	(4)	(4)	(4)	(4)	(4)	(10)

mal values in all categories. The notable increase above normal in oxygen consumption is perhaps due to greater muscular activity than during the initial period of observation under morphine.

Comparison of the foregoing data with those in dogs cooled after the induction of shock (table II) reveals several points of interest. The fall in oxygen consumption and cardiac output is appreciably less than in the precooled dog and the respiratory depression is not nearly so steep. The higher level of metabolic activity, as reflected in the higher oxygen consumption, suggests that when the circulation is already defective external cooling may fail to reduce the temperature of some of the deeper tissues to the same level as in the precooled animal.

THE CIRCULATION DURING REWARMING

HENRY SWAN

I should like to discuss the state of the circulation during the period of rewarming and the first three hours thereafter. On the basis of clinical and laboratory experience, it is our opinion that acute circulatory insufficiency exists during at least a part of this time. The experiments I wish to present involve mild hypothermia. Dogs were surface-cooled to 30° C. (rectal), maintained thus for one hour, rewarmed to normal temperature and studied thereafter each hour for the next three hours.

A typical protocol is shown in figure 1, where it can be seen that femoral arterial pressure is low following cooling. During rewarming there is a rise in pressure but control values are not marked by the end of three hours. Cardiac rate post-warming is elevated, as is total peripheral resistance. Following warming total oxygen consumption and A-V oxygen difference tend to be elevated. Cardiac index is below normal at this time.

So here we have a dog with a rapid heart rate, a low blood pressure in spite of the high peripheral resistance, who has not been able to raise his cardiac output, and yet has a high oxygen consumption. He is alive only because, as Dr. D'Amato pointed out, he is able to extract a lot more oxygen from the blood. I maintain that, at this stage, the animal has an insufficient circulation for his needs.

If the ratio between cardiac output and oxygen consumption during cooling and during the cool state is plotted, there is found a linear relationship (fig. 2). The line is the same for the control normothermic period and the cool state. In other words the normothermia and the hypothermia plots fall pretty much on the same line. Under these conditions we feel that the circulation is adequate.

To contrast with that, figure 3 shows the slant of the line in the post-hypothermic state, in which the cardiac output has failed to maintain pace.

Figure 4 illustrates the oxygen consumption of the left ventricle as measured by A-V oxygen differences across the coronary circulation. The oxygen consumption decreases and even three hours later it has not returned to the previous level.

Finally, the efficiency of the left ventricle falls off during the cool state (fig. 4).

It is not proper, therefore, to state that if an animal is cooled and rewarmed, he returns to physiologic normality. I think it is more proper to state that for a period of at least three hours after mild hypothermia in a dog (whose respiration has been unsupported) there is a state of circulatory insufficiency.

DISCUSSION

Dr. R. O. Heimbecker: We have noted complications during rewarming which were similar to those described by Dr. Swan. We believe that these are due to too rapid warming. As the result of excess surface heat peripheral arteriolar dilation results, peripheral resistance is lowered and a shock-like state develops. Removal of some of the surface heat must be a part of therapy.

Dr. Jacob Fine: In Dr. Friedman's experiments on cooled dogs subjected to hemorrhagic shock the effects of morphine and ether were pretty well dissipated before

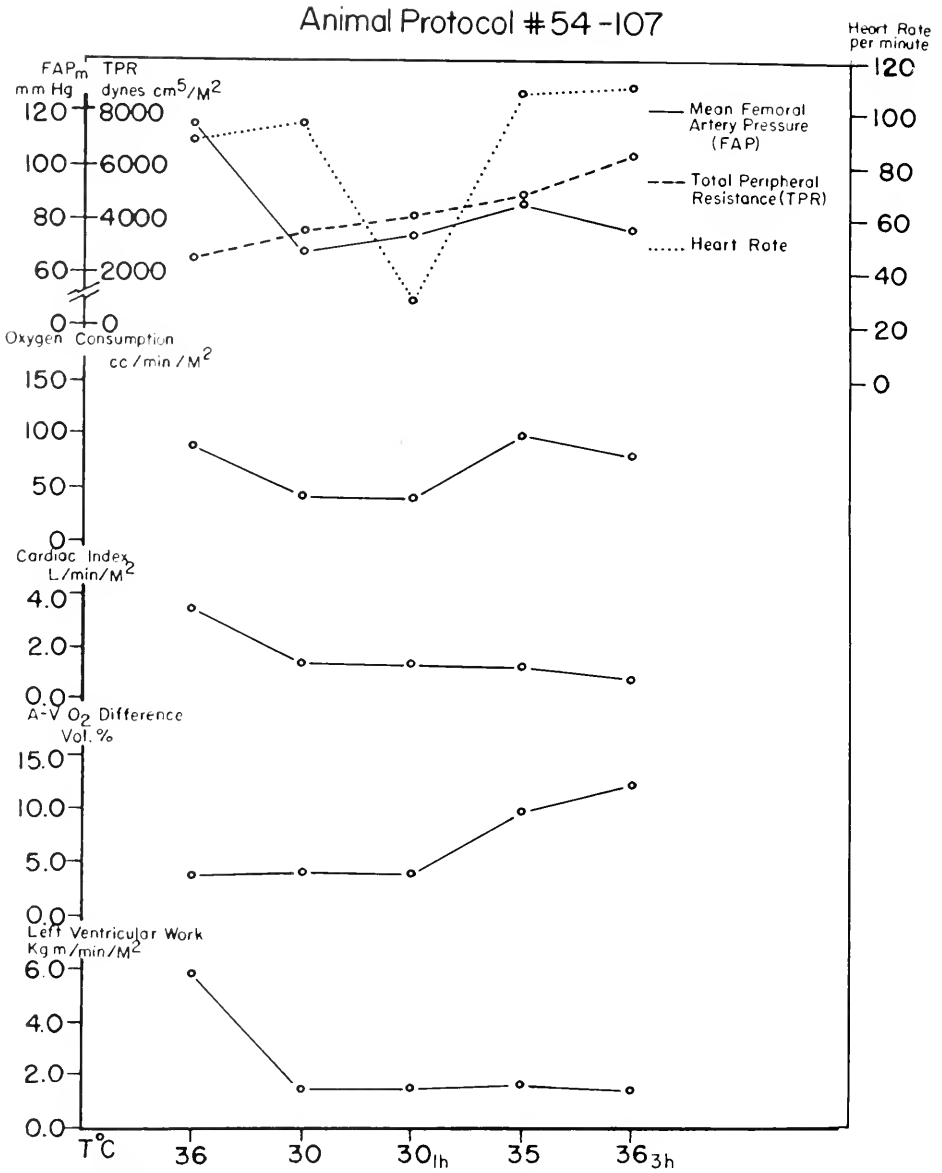


FIG. 1.—Physiologic changes during hypothermia and on rewarming in a typical experiment. Three hours after rewarming the animal is in circulatory failure.

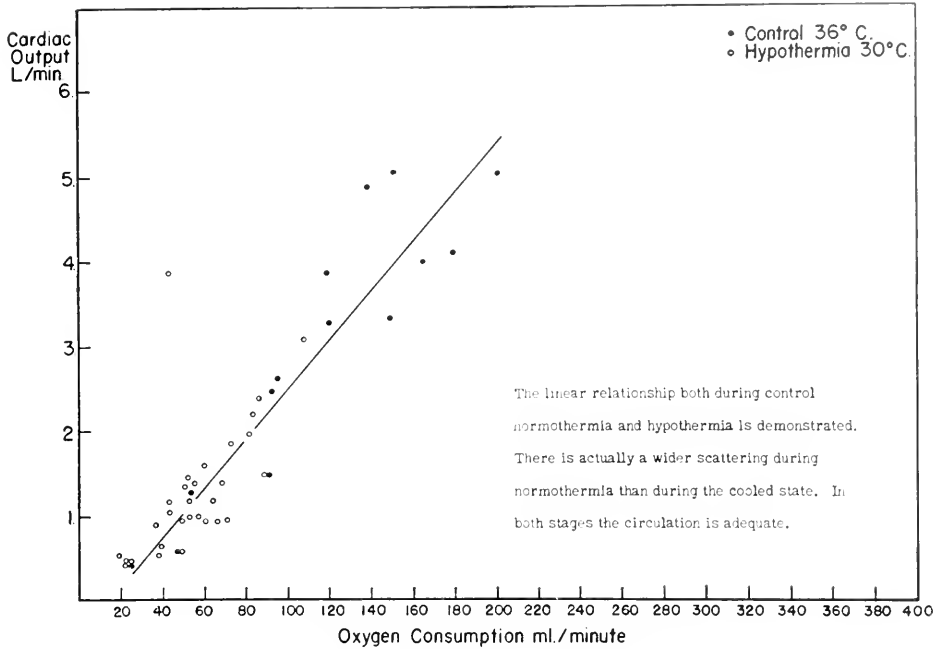


Fig. 2.—Relation between cardiac output and oxygen consumption during cooling.

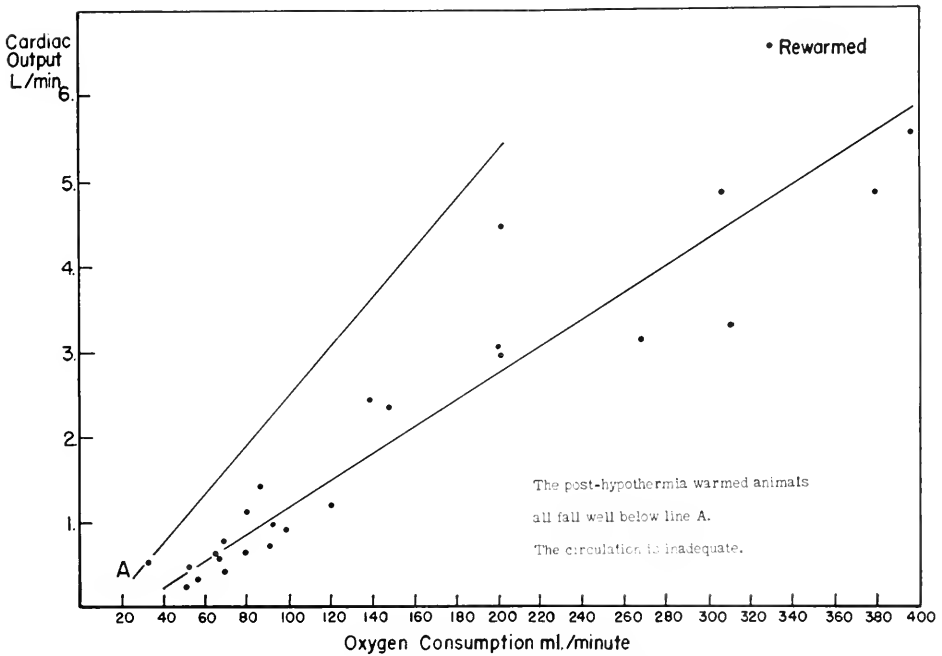


Fig. 3.—Relation between cardiac output and oxygen consumption during rewarming. Line A is derived from Fig. 2.

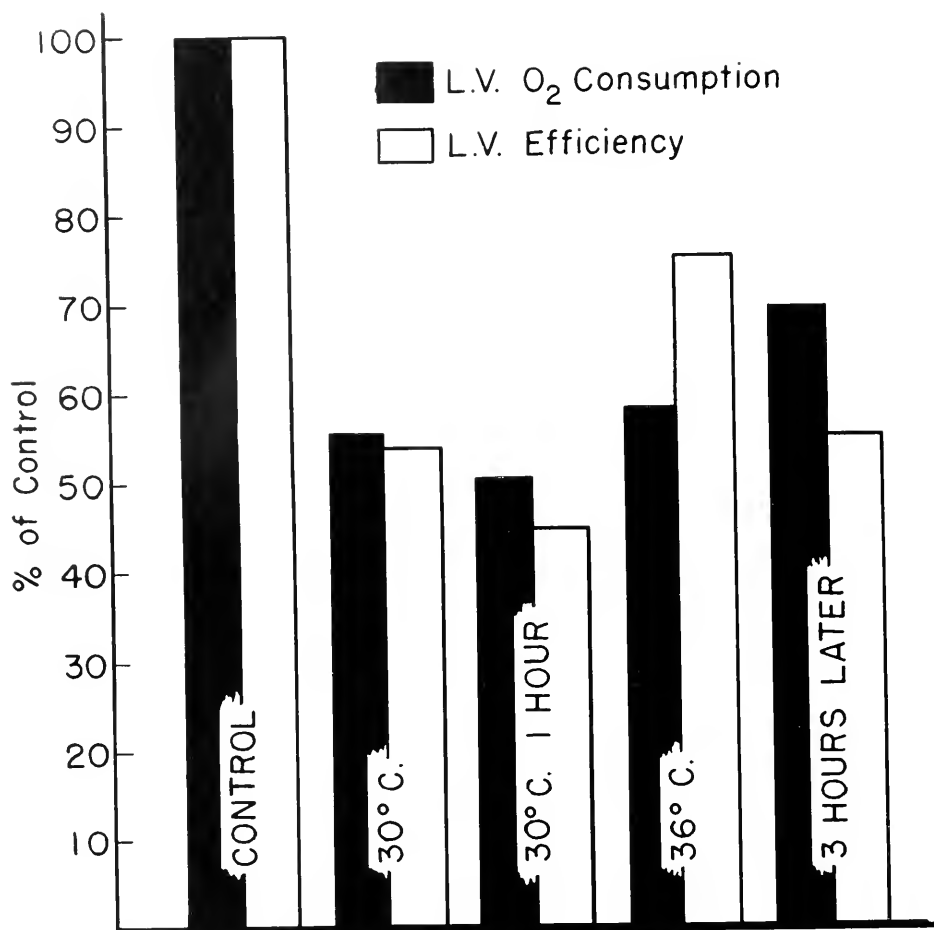


FIG. 4.—Oxygen consumption of left ventricle measured by A-V oxygen differences across the coronary circulation.

rewarming was started so that the animal could be considered virtually free of the effects of drugs and of any significant surgical stress. As he stated, no special hazards during the warming period were encountered except that transfusion induced ventricular fibrillation if performed rapidly, but not if performed slowly. This suggests a possible cardiac disability, in spite of which recovery occurred.

Dr. Swan's data on patients suggest a more hazardous state of the circulation during the rewarming stage. Is this, like Dr. Millikan's observations on the myocardium, a direct consequence of the hypothermia or of some other factor? What bearing has the surgical procedure itself or the anesthetic upon them? Would the cooled heart during the return toward normal temperature be any freer of these dangers if the effects of drugs used before and during operation including the anesthetic were excluded?

CORONARY BLOOD FLOW DURING HYPOTHERMIA

R. M. BERNE

Dr. D'Amato referred to our experiments in which we found that coronary blood flow was fairly well maintained in the hypothermic state at a time when aortic pressure had reached very low levels. This is illustrated in figure 1, where the coronary blood flow is plotted against perfusion pressure (mean aortic pressure) during progressive hypothermia. At a temperature of 21° C. when perfusion pressure had reached 42 mm. Hg. and coronary blood flow was 11 cc. per minute we elevated perfusion pressure by means of a mechanical pump (line B) and observed that at comparable pressures coronary blood flow was in excess of that noted during the induction of hypothermia. We have extended these studies in an attempt to determine the cause of this lowered resistance in the coronary bed during hypothermia.

Two types of experiments were carried out. In one we cooled the blood entering the common left coronary artery or its circumflex branch and made observations on flow, and in the other we made plastic coronary flow measurements in hypothermia. Figure 2 depicts the effect of alternately cooling and warming the blood perfusing the left coronary artery in a dog with normal body temperature. From top to bottom, we have perfusion pressure, coronary blood flow and the temperature of the blood entering the circumflex branch of the left coronary artery, all plotted against time.

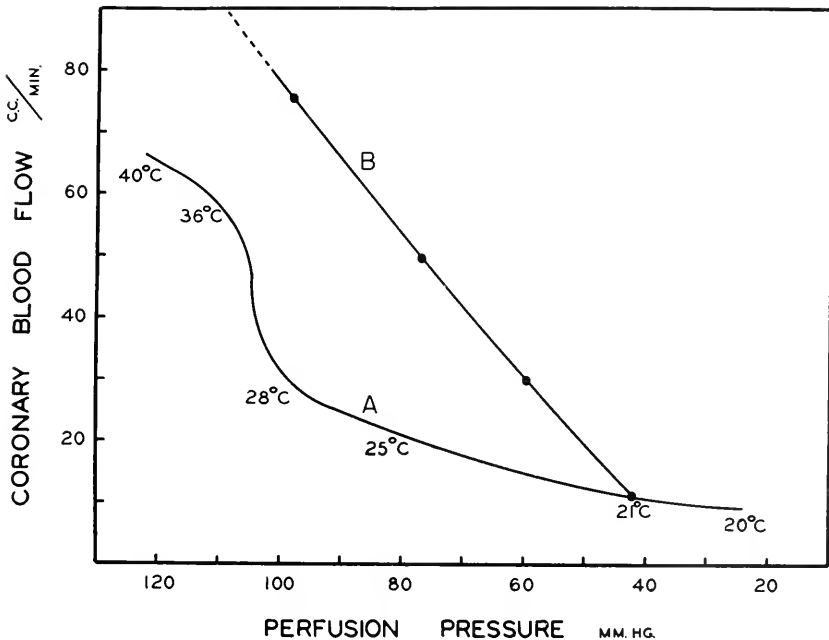


FIG. 1.—Effect of increasing coronary perfusion pressure in severe hypothermia. Curve A is the pressure/flow curve in progressive hypothermia. Curve B represents the flows obtained at blood temperature of 21° C. when perfusion pressure was artificially elevated.

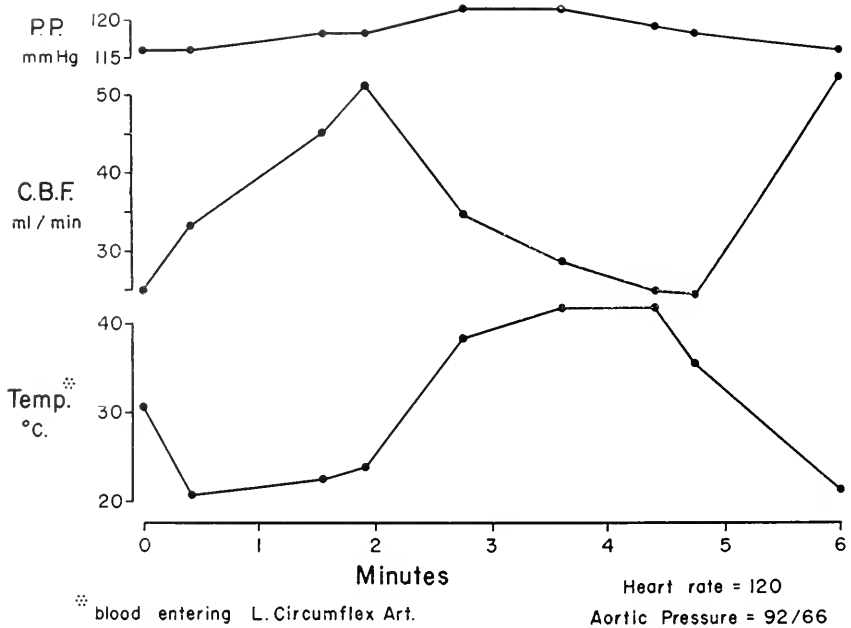


FIG. 2.—Effect of changing temperature of blood perfusing the left circumflex coronary artery on coronary blood flow in the normothermic open chest dog. Perfusion pressure was kept relatively constant by a pump-perfusion system.

The heart rate was 120 beats per minute and the aortic pressure 92/66 millimeters of mercury throughout the experiment. Perfusion pressure was kept relatively constant during the experiment by means of a pump-perfusion system. Lowering the temperature of the blood perfusing the left coronary artery produced an increase in flow which returned to control levels when the blood temperature was increased to normal values.

Similar experiments were performed with the fibrillating dog heart and as can be seen in figure 3 an inverse relationship exists between the temperature of the blood reaching the left coronary and the rate of flow in this vessel. The increase in flow that we observed with decrease in blood temperature did not occur immediately after the temperature change. In fact, we missed it at first because we changed the temperature too rapidly. However, if we waited one to two minutes after altering the blood temperature, changes in flow would occur with great regularity.

We do not know the mechanism by which this decrease in resistance in the coronary bed is produced with cold but can only surmise, as did Anrep and his colleagues a number of years ago, that it is a direct effect of cold on the vessel wall.

In these experiments in which the left coronary arterial blood was cooled we measured the oxygen consumption of the myocardium and found it to be essentially constant.

We made an interesting and rather puzzling observation on the plasma potassium levels across the heart during periods when the left coronary artery blood was cooled. In each of these experiments the potassium concentration was lower in the

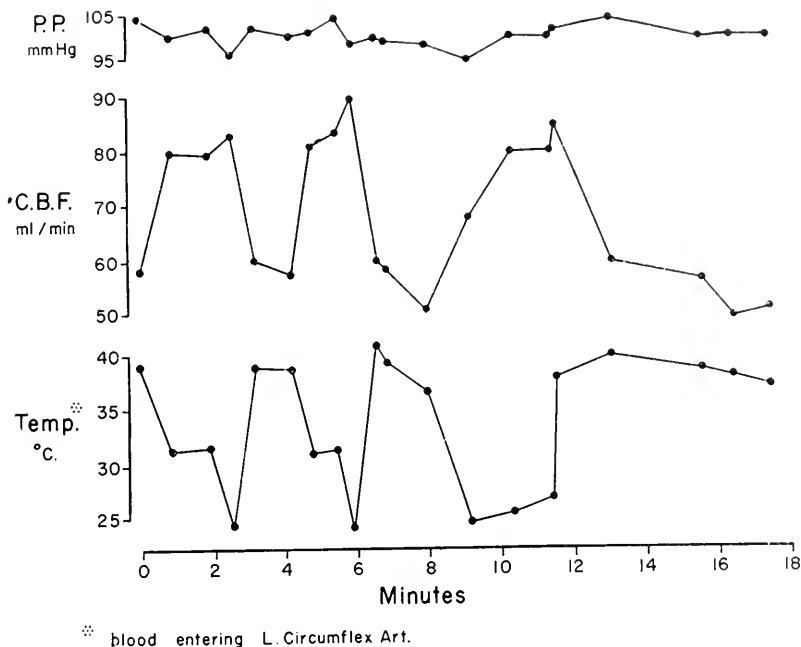


FIG. 3.—Effect of changing temperature of blood perfusing the left circumflex coronary artery on coronary blood flow in a dog fibrillating-heart preparation. Perfusion pressure was kept relatively constant by a pump-perfusion system.

coronary sinus blood than in the arterial blood. The average decrease of potassium in coronary sinus blood in 11 experiments was 15 per cent. In the control periods in which the left coronary inflow was kept at normal body temperature the coronary sinus blood potassium concentration equaled that found in the arterial blood. In two experiments in which the whole animal was cooled the potassium level in the cardiac venous blood was below the arterial blood concentration, a finding similar to that of Dr. Swan.

Another interesting observation we made in this series of experiments was that in seven out of eleven normothermic dogs ventricular fibrillation occurred when blood entering the left coronary artery was cooled.

The second type of experiment we did to shed some light on the factors influencing coronary resistance in hypothermia was to make phasic flow measurements in the circumflex coronary artery of the dog in normothermia and hypothermia. At present we have done only a few such experiments. The bristle flowmeter of Brecher and Praglin was used to make these flow measurements.

We were interested in finding out whether the prolonged period of isometric relaxation that obtains in hypothermia restricts coronary blood flow. Figure 4 depicts records of simultaneously recorded aortic, left ventricular, and left atrial pressure curves in progressive hypothermia. In part A at a temperature of 40° C. isometric relaxation is brief, 0.04 seconds, whereas in part B at 20.5° C. it occupies a significant portion of the cardiac cycle, 0.52 seconds. However, in this experiment the duration of systole increased from 0.18 to 1.00 seconds and the question is how

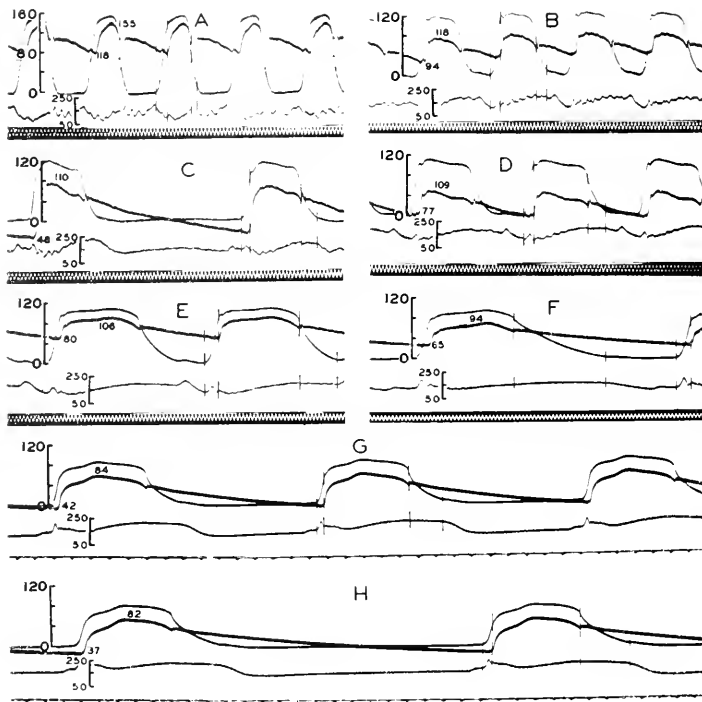


FIG. 4.—Effect of hypothermia on ventricular (upper curve), aortic (middle curve with incisura), and left atrial (lower curve) pressure pulses in the same dog. A, control heart blood temperature 40°C .; B, 33.5°C .; C, 33.5°C . with peripheral right vagus stimulation; D, 27.5°C .; E, 26°C .; F, 23°C .; G, 22°C .; H, 20.5°C . Ventricular and aortic pressures in millimeters Hg. Left atrial pressure in millimeters saline. Time scale records A through F, 0.02 second; records G and H, 0.20 second.

much an impedance to inflow in the coronary bed is caused by the slow ventricular relaxation and how much by the prolonged systole.

In figure 5 we have records of an aortic pressure curve with a phasic coronary flow curve beneath. These records were obtained from the same dog at 36°C . (No. I) and 23°C . (No. II). In normothermia we observe a sharp decrease in flow with some backflow in early systole followed by a slight rise in late systole. In early diastole there is a sharp increase in flow followed by a decrease that is associated with the fall in aortic pressure. In hypothermia there is also a slight reversal of flow during systole followed by a slight rise and fall. The latter corresponds to the dip in pressure caused by the standing wave. However, in diastole there is a gradual and continuous increase in coronary blood flow during ventricular relaxation (line B-C). It is only shortly before the next systole that flow follows the aortic pressure curve. It appears that this rising coronary flow in the face of a falling aortic pressure is due to the gradual reduction in extravascular compression as the

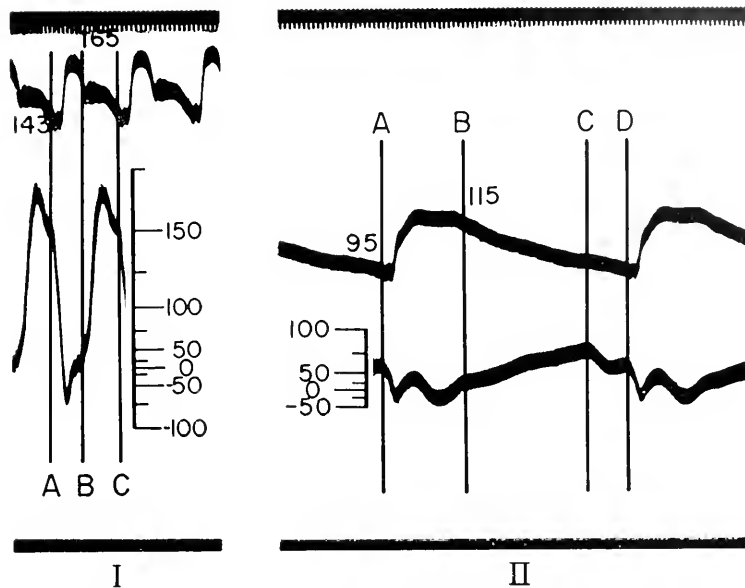


FIG. 5.—Aortic pressure curve (upper) and phasic coronary artery flow curve in the same dog at 36° C. (I) and 23° C. (II). Systole from A-B in both records. Diastole B-C in record I and B-D in record II. Aortic pressures in mm. Hg. Flow in ml. per minute. Time—0.02-second intervals.

ventricles slowly relax. It is interesting to note that 66 per cent of the coronary inflow occurs during isometric relaxation in hypothermia despite the decreasing perfusion pressure and the prolonged period of extravascular compression.

THE EFFECT OF HYPOTHERMIA ON PITUITARY ACTH RELEASE AND ON ADRENAL CORTICAL AND MEDULLARY SECRETION IN THE DOG*

D. M. HUME, R. H. EGDAHL† AND D. H. NELSON

Some studies on adrenal cortical function in hypothermia are available in the literature. Khalil,⁸ using adrenal ascorbic acid depletion, reported that the adrenal was responsive to ACTH stimulation during hypothermia, although the response was greatly decreased. Egdahl *et al.*,^{2, 3} found that adrenal venous blood 17-hydroxycorticosteroid secretion was markedly reduced in hypothermia, and similar results were obtained by Ganong *et al.*⁵

The present brief report concerns work done by our group on blood ACTH levels, adrenal corticosteroid secretion, and epinephrine and norepinephrine output in hypothermia. Most of this work has been or will be reported in more detail elsewhere.

Methods. The hypothermia studies were carried out, for the most part, as acute experiments on dogs which were traumatized under ether anesthesia and then cooled. Adrenal venous blood samples were obtained by the method of Hume and Nelson.⁶ This consisted of placing a cannula in the lateral aspect of the lumbo-adrenal vein and briefly occluding the adrenal vein at its junction with the vena cava. A polyethylene snare was used so that intermittent occlusion of the vein could be produced externally whenever it was desired to obtain a sample. During the periods of occlusion all of the adrenal blood flowed out the cannula and was collected in graduated centrifuge tubes. The adrenal venous blood was assayed for 17-hydroxycorticosteroid content by the method of Nelson and Samuels.⁹

Arterial blood ACTH content was determined by the method of Nelson and Hume.¹⁰ In this technique the corticosteroidogenic capacity of the peripheral blood ACTH is tested in the hypophysectomized dog. The blood ACTH was determined before the induction of hypothermia, during hypothermia, and after rewarming.

Adrenal venous blood epinephrine and norepinephrine levels were measured by the method of Aronow.⁴

Hypothermia was induced by ice water immersion, air cooling, or by cooling an external vascular shunt. The animals were cooled to 21°–28° C. Some of the animals received a constant intravenous drip of ACTH at a rate of 80 milliunits per minute during the production of hypothermia. In some experiments the adrenal was cooled locally while the animal remained normothermic, and in others the animals were allowed to become hypothermic while the systemic blood pressure was maintained with a continuous infusion of norepinephrine. Adrenal function was also assessed in normothermic unanesthetized dogs exposed to cold at –10° C., –48° C. and –78° C. In these dogs the adrenal cannula was placed under sterile

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conditions and the dog was allowed to recover. The animal was then exposed to cold three or four days later, when his adrenal corticosteroid secretion had reached basal levels. Three such dogs were anesthetized with nembutal and rendered hypothermic in an ice water bath, without the addition of any trauma.

Results. The results may be summarized as follows:

1. Pituitary ACTH secretion in the traumatized animal decreases markedly during hypothermia, and resumes high levels again on rewarming^{2, 7} (fig. 1).
2. Adrenal 17-hydroxycorticoid production in the traumatized animal is decreased to very low levels during hypothermia and returns rapidly to normal on rewarming.^{2, 3}
3. Corticoid output is decreased in hypothermia even if the systemic blood pressure is maintained at near normal levels.^{2, 3}
4. The adrenal is unresponsive to large doses of ACTH while the animal is hypothermic, and the response rapidly returns on rewarming.^{2, 3}
5. Cold appears to act directly on adrenal enzyme systems. When local cold is applied to the adrenal and the animal remains normothermic the adrenal corticosteroid secretion is sharply reduced.^{2, 3}

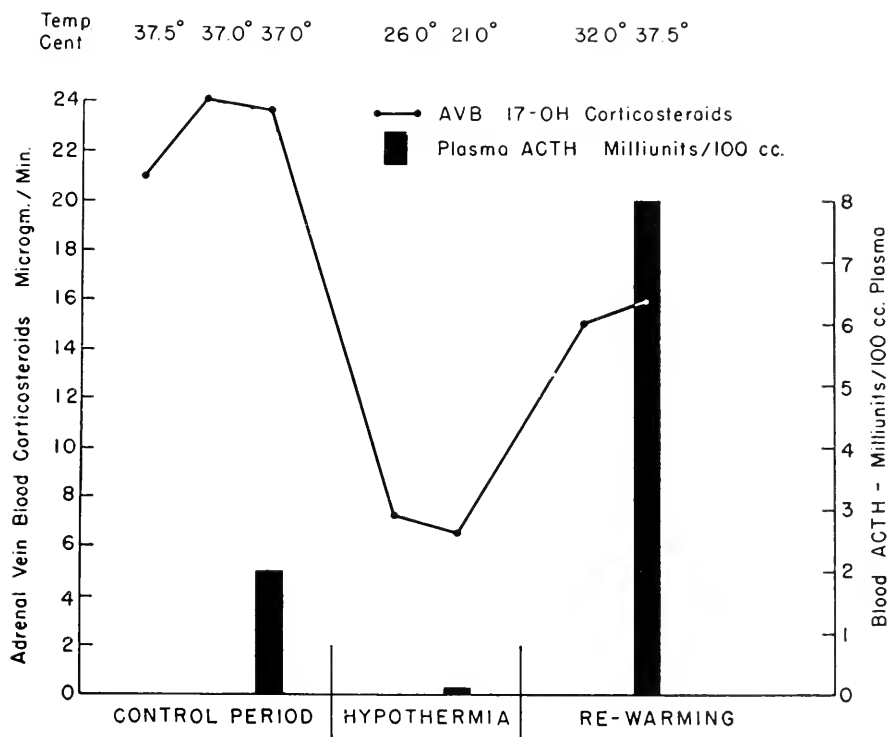


FIG. 1.—The blood ACTH levels and adrenal vein blood 17-hydroxycorticosteroid output in a dog traumatized under ether anesthesia and then subjected to hypothermia. It may be seen that there is a marked depression of corticosteroid secretion during hypothermia, with an increase again after rewarming. Blood ACTH, which is at a measurable level before the induction of hypothermia, becomes too low to measure during hypothermia. On rewarming, ACTH is again present in easily detectable amounts.

6. Corticoid secretion is reduced in most dogs to less than 25 per cent of normal at temperatures ranging from 25° to 28° C. In a few cases, suppression of corticoid secretion to this extent did not occur until the temperature was reduced 22°–23° C.^{2, 3, 7}

7. Similar results were obtained whether hypothermia was induced by ice water immersion, air cooling, or by cooling an external vascular shunt.

8. The exposure of normal dogs to temperatures of –10° C. for periods up to 34 hours did not produce hypothermia or adrenal activation, and did not alter adrenal response to ACTH.¹¹ The exposure to –48° or –78° C. produced temporary adrenal activation, but did not produce hypothermia or alteration in adrenal function.⁴

9. The induction of hypothermia under nembutal anesthesia without surgical trauma produced no significant corticosteroid secretion.³

10. Hypothermia to 26° C. produces a 10-fold, and to 21° C. a 100-fold decrease in the secretion of epinephrine and norepinephrine by the adrenal medulla⁷ (fig. 2).

Conclusions. 1. Trauma under ether anesthesia produces marked increases in pituitary ACTH and adrenal corticoid secretion. The induction of hypothermia greatly depresses the output of these hormones. Pre-hypothermia ACTH and corticoid levels are again noted when the animal is rewarmed. Adrenal sensitivity

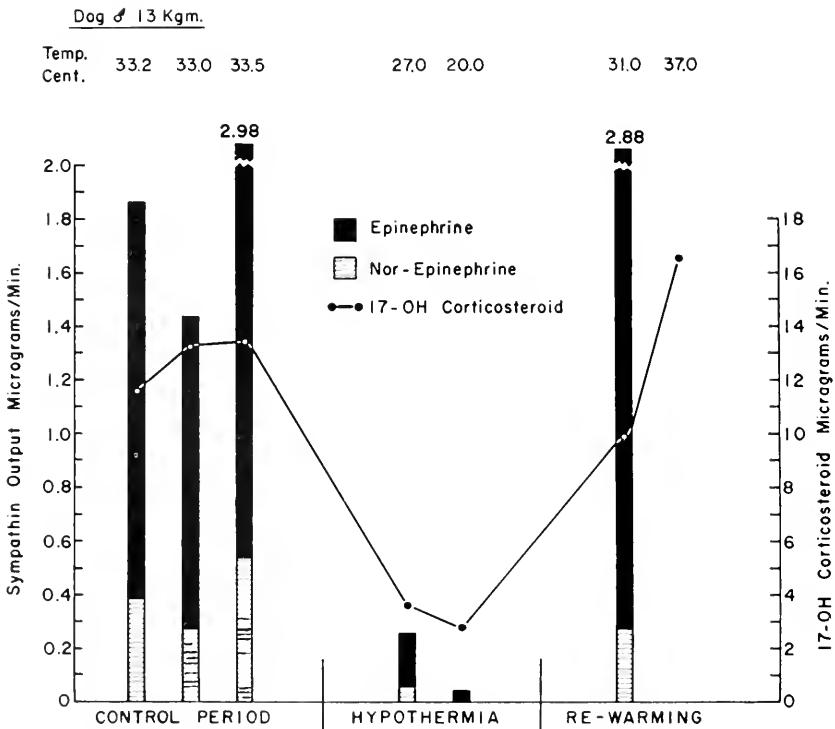


FIG. 2.—Epinephrine, norepinephrine, and corticoid secretion in the adrenal venous blood of the traumatized dog before, during, and after the induction of hypothermia. A very marked decrease in epinephrine and norepinephrine output occurs during hypothermia.

to exogenous ACTH is likewise markedly reduced, apparently as a direct effect of lowered temperature on the adrenal cortical cells.

2. Adrenal medullary secretion of epinephrine and norepinephrine is sharply reduced in hypothermia.

3. By contrast, cold exposure without the development of hypothermia does not alter adrenal responsiveness to ACTH.

4. The induction of hypothermia *per se* under anesthesia, but in the absence of trauma, did not act at any time as a stimulant to pituitary-adrenocortical secretion.

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DISCUSSION

Dr. A. D. Keller: We have made observations of dogs having graded hypophysectomies. These correlate well with Dr. Hume's observations. The hypophysectomized dog, regardless of the magnitude of the hypophysial deficiencies, tolerates cold without an adrenal insufficiency crisis being precipitated. The dog that is almost totally hypophysectomized, so that there is practically no adrenal cortex left, has such a reduced resistance to cold that on exposure a progressive hypothermia develops.

Dr. M. E. De Bakey: I wonder if Dr. Hume would tell us what was the body temperature of the dogs who were exposed to -78°C .

Dr. Hume: They remained normal.

There are two other points which are perhaps worth noting. One is that when you measure peripheral corticoid levels, you are measuring not only adrenal corticoid production but also peripheral destruction and excretion. Both of these factors are reduced under hypothermia. Although corticoids are being produced in smaller amounts than under normal circumstances, they are also being destroyed more slowly, because the ability of the liver to conjugate, and thus inactivate, them is

very greatly reduced. It is difficult, therefore, to get an accurate idea of adrenal steroid production by measuring peripheral levels of corticoids in hypothermia.

The second point is that the dog appears uniquely able to withstand severe cold. Cold at -10°C . does not increase corticoid production, and the adrenalectomized dog can withstand this temperature without ill effect. Apparently even the induction of hypothermia under anesthesia is not a stimulus to the hypothalamic-pituitary-adrenal system in this species.

Dr. J. R. Pappenheimer: Is chemical therapy a good or bad thing? Is there any difference in the survival of animals if they are given supplementary doses of drugs?

Dr. Moore: Thank you, Dr. Pappenheimer.

In answer to your question, we may anticipate a bit. In the next talk, Dr. Bernhard will show an anesthesia chart which is one item in a fairly broad study that we are now doing on the effect of anesthetic agents on the stress mechanism, or whatever you want to call it, in man. I also expect that, in the discussion to follow, Dr. Swan will refer to his studies on the effect of drugs in hypothermic subjects. Some of our work has been along the same lines. During the past three years we have found that pentothal, nitrous oxide, curare, cyclopropane—spinal and local—are all of interest in that they provide a very mild or practically zero stimulus to adrenocortical secretion as measured in the peripheral blood.

Cyclopropane is interesting since it does not stimulate the adrenocortical system, but information we have obtained in collaboration with Dr. Aronow indicates that it does stimulate the epinephrine-norepinephrine system, and it is a very strong stimulant to anti-diuresis. So we are beginning to see dissociations in various types of endocrine stimulation with different agents. "Stress" covers a multitude of phenomena. Ether leads the list in causing a rise in the serum corticoids.

As a patient recovers from a surgical operation which involves a big cross-sectional tissue trauma, he will have a secondary rise in serum corticoids as he comes out of the anesthetic agent. The obvious explanation has to do with pain. We can't prove that that is the case.

In the experiments planned by Dr. Swan it will be interesting to see what happens. If the animal is traumatized under hypothermia and then comes out of both the hypothermia and the anesthesia, it would be my prediction he would then show a peripheral rise in 17-hydroxysteroids.

Two or three things can be said about the teleology of all this. First, if an animal is incapable of summoning any endocrine response to trauma, the animal does very poorly. Second, there are circumstances in which the endocrine response to trauma appears to be massive, as evidenced either in terms of nitrogen-potassium changes, or measurements of corticoids in blood or urine, and those patients seem to be very sick as if something "overshot." Third, it seems that we are seeing a "middle ground" in hypothermia which does not inhibit the adrenal to the point that the patient or the animal responds like an Addisonian, and yet produces a modified response. It is probably a good thing that the adrenal activity is not completely inhibited, but is somewhat restrained. I have been impressed by this in the ten or twelve patients we have observed. Under hypothermia, an amount of operative trauma which the next day should produce a very sick-looking patient instead produces a mild but normal posttraumatic reaction.

THE EFFECT OF HYPOTHERMIA ON THE PERIPHERAL SERUM LEVELS OF FREE 17-HYDROXYCORTICOIDS IN THE DOG, AND IN MAN

WILLIAM F. BERNHARD

The experimental investigation and clinical application of hypothermia in the care of surgical patients has become increasingly important. Technical advances have predominated so far, while the metabolic response of the organism to operative stress in hypothermia has not as yet been clearly defined. Recently, direct methods for measurement of 17-hydroxycorticoids were made available, and studies have been carried out in normal individuals, and in patients during and after surgery at normal body temperature. Utilizing these techniques in the experimental animal, data have been presented demonstrating an inhibition of the adrenocortical 17-hydroxycorticoid output at low temperatures. It was found that there was a marked reduction in adrenal venous blood flow without a compensatory rise in corticoid concentration, and thus a low corticoid minute output resulted.

The level of 17-hydroxycorticoids in the arterial blood plays a major role in regulating the metabolic response of the organism. This level is determined not only by adrenocortical production of these substances, but also by their rate of utilization, conjugation, and excretion. Concomitant depression of these three processes may be anticipated in hypothermia, since cooling depresses tissue metabolism throughout the organism.

Peripheral 17-hydroxycorticoids in the dog. A group of adult mongrel dogs were anesthetized with pentobarbital sodium, intubated, and hyperventilated with a positive pressure apparatus. A polyvinyl cannula was inserted into a femoral artery, permitting arterial blood sampling. An initial laparotomy was then carried out and closed, following which the first peripheral blood sample was obtained. This represented the control level from a surgically stressed normal animal. Hypothermia was then induced and after a period of stabilization, a second laparotomy was performed and the second arterial blood sample collected. In all animals the inferior mesenteric, superior mesenteric, celiac axis, hepatic artery and portal vein were then isolated and occluded with bulldog clamps, permitting complete exclusion of the liver from the circulation. The occlusions were maintained for one hour and then the third arterial blood sample was obtained. The clamps were then released, the incision closed and the animals rewarmed in a warm water bath. The final arterial blood sample was obtained when the body temperature returned to normal. Free plasma 17-hydroxycorticoids were determined by the method of Silber and Porter.

The mean 17-hydroxycorticoid levels (fig. 1) in the arterial plasma of the dogs studied revealed a plasma corticoid level of 17.8 ± 1.5 gamma per cent after the control laparotomy at normal body temperature. Following the induction of hypothermia and stabilization of the animal's temperature at 25° C. a second laparotomy was performed. The mean corticoid concentration at the end of this procedure was 24.8 ± 3.5 gamma per cent. After one hour of exclusion of the liver from the cir-

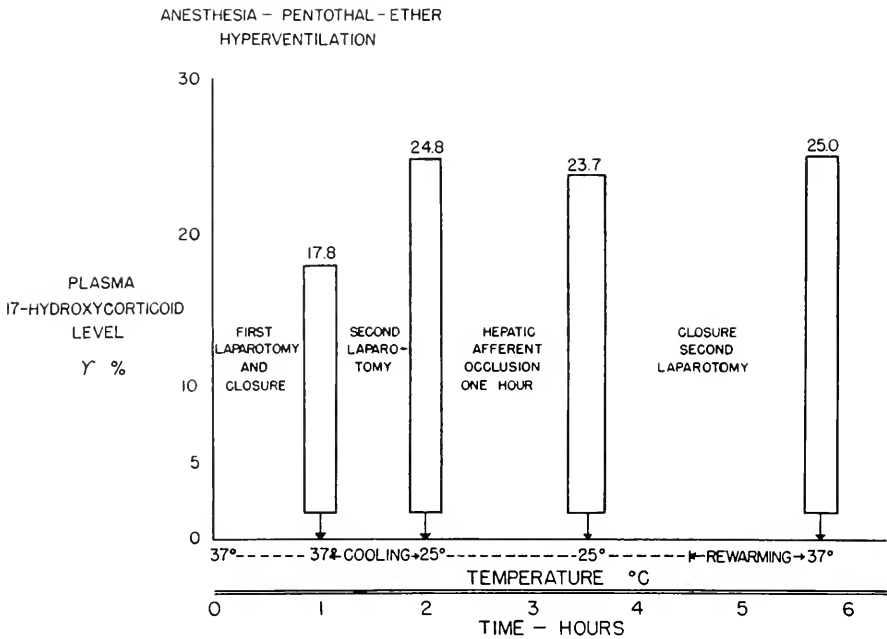


FIG. 1.

ulation, the level was 23.7 ± 3.6 gamma per cent. Finally, after rewarming to normal body temperature, the level was 25.0 ± 2.8 gamma per cent.

The half life of hydrocortisone in the blood of the normal animal is less than one hour, the hormone being conjugated by the liver, excreted in the urine and possibly utilized by the tissues. The fact that the arterial corticoid levels in the animals studied increased rather than decreased after two hours of cooling and further trauma suggests that depression of these mechanisms is equal to or greater than the fall in adrenocortical production of the hormones. This conclusion was strengthened by the fact that exclusion of the liver from the general circulation failed to produce a change in arterial corticoid levels. If the liver were conjugating steroids at a normal rate in the hypothermic animal, an appreciable fall in plasma corticoid levels would have resulted. It is evident, therefore, that the marked reduction in adrenocortical hormone production in hypothermia is more than balanced by the concomitant depression of mechanisms which normally tend to reduce this level.

Peripheral 17-hydroxycorticoids in man. A small group of patients undergoing surgery with hypothermia were also studied (two examples shown in figures 2 and 3). Peripheral venous blood samples were obtained before, during and for 7 to 13 days following surgery. Blood was collected in dry test tubes, allowed to clot, and the serum removed. Samples were stored in a deep freeze until analyzed, at which time the corticoid determinations were carried out by the method of Nelson and Samuels.

The normal range for peripheral 17-hydroxycorticoids in this laboratory is 15 to 18 gamma per cent with a mean of 12 gamma per cent. Additional patients under-

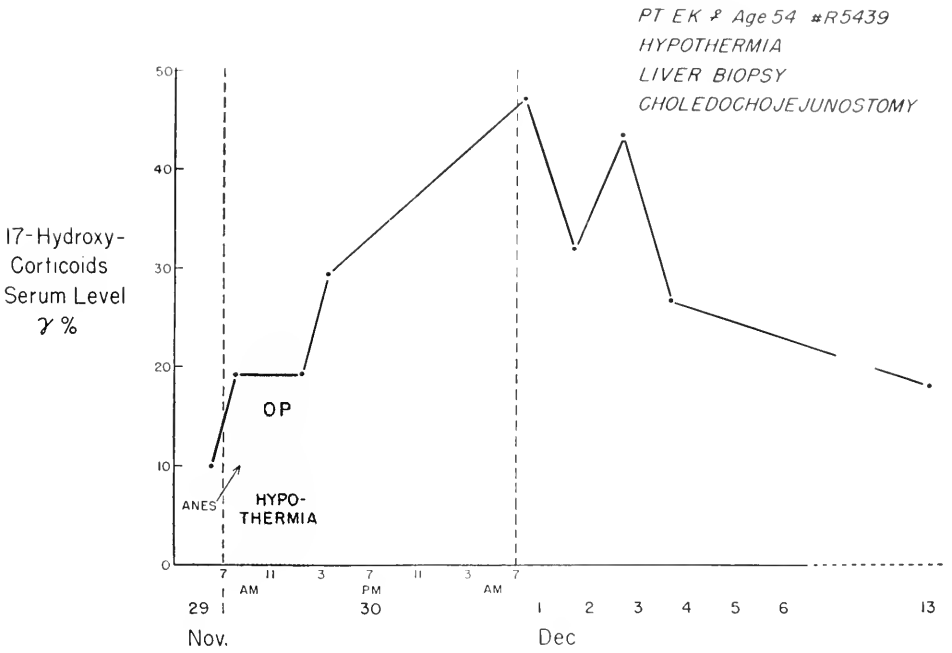


FIG. 2.

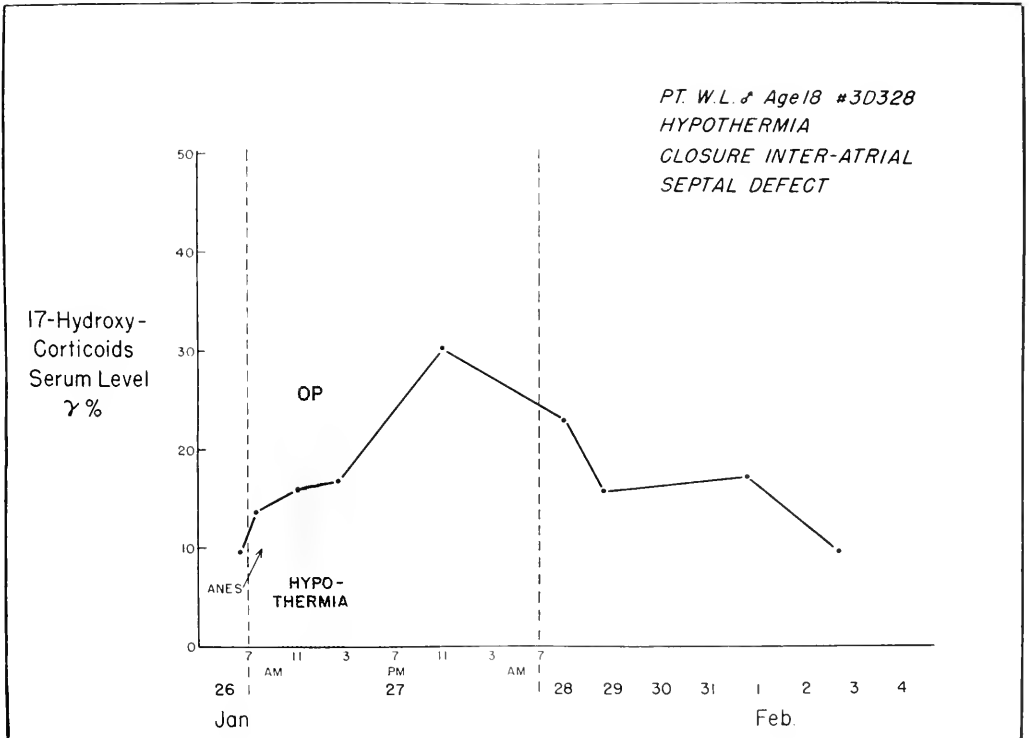


FIG. 3.

going surgery under general anesthesia at normal body temperature have been included in this study for comparison with the hypothermia cases. The peripheral 17-hydroxycorticoid levels in these patients were followed as a part of a study of endocrine responses to anesthesia.

In the patients studied, the peripheral corticoids also revealed a constant level during the course of surgery under hypothermia. These steroid values are different from those obtained in the experimental animal in that the patients were not subjected to surgical stress prior to cooling. In these cases, the stress response provoked by anesthesia *per se* was not marked. With cooling, and during extensive surgery, there was no rise in corticoid levels. Furthermore, the values remained within the normal range for several hours after completion of rewarming. A post-surgical rise did occur as revealed by samples obtained within the next 12 hours. However, in all cases, the magnitude of response was less than that expected from the degree of surgical trauma that took place.

The usual pattern of 17-hydroxycorticoids during anesthesia and surgery at normal body temperature is characterized by an initial steep rise with induction of anesthesia, reaching levels 4 to 6 times the resting value. Peak levels are generally reached within 2 to 3 hours following the conclusion of the operation. Thereafter, a sharp fall occurs with normal values again present 24 to 73 hours later. This type of response has been recorded in numerous patients undergoing abdominal surgery with pentothal-ether anesthesia.

Summary. 1. The peripheral arterial level of plasma 17-hydroxycorticoids has been presented in dogs subjected to laparotomy before and during hypothermia. The effect of exclusion of the liver from the circulation was determined.

2. The peripheral venous corticoid levels were followed in a small group of patients subjected to surgery under hypothermia and compared with the response to surgery in normothermic patients.

3. The dogs and patients revealed constant peripheral corticoid levels with extensive surgical trauma during hypothermia.

4. Hypothermia with the concomitant reduction of body metabolism simultaneously depresses production and conjugation of the steroid hormones and to a similar degree.

5. A post-surgical rise in peripheral corticoids does occur but the magnitude is less than that expected with comparable major surgery performed at normal temperature.

6. The function of the liver in conjugation of steroids during hypothermia is depressed.

7. Immersion cooling does not provoke a stress response as measured by peripheral 17-hydroxycorticoids.

DISCUSSION

Dr. Jean Cahn: The problem is to compare in different methods of hypothermia the reaction of the adrenal and of the pituitary during stress reaction or during cooling of the body.

Here is the evolution of the ascorbic acid level of the adrenal in rats during artificial hibernation. You could see during the presentation of the paper on corticosteroid secretion of the adrenals during the cooling of the body that the initial re-

action of the adrenal is a fall in the corticosteroid secretion. The relationship between the ascorbic acid level and the corticosteroid secretion of the adrenal is not clear. But in surface cooling the initial reaction of the adrenal to the cooling is a fall of about 28 per cent in the adrenal ascorbic acid level, and this level remains stable during all the period of the cooling.

It is interesting to compare the phenomenon in artificial hibernation. Immediately after the injection of drugs such as chlorpromazine or hydergine in association with phenergan and demerol, one observes a fall in ascorbic acid level, 30 to 50 per cent, approximately. Immediately after the injection of the drugs the body temperature begins to fall. During the fall in the body temperature we found a gradual return to normal of the adrenal ascorbic acid; the significance of this fact is that cold is not a stress in artificial hibernation. There is a very different reaction in surface cooling. During all the time of cooling we have a 28 per cent lower adrenal ascorbic level.

Figure 1 shows the adrenal ascorbic acid level in artificial hibernation after five

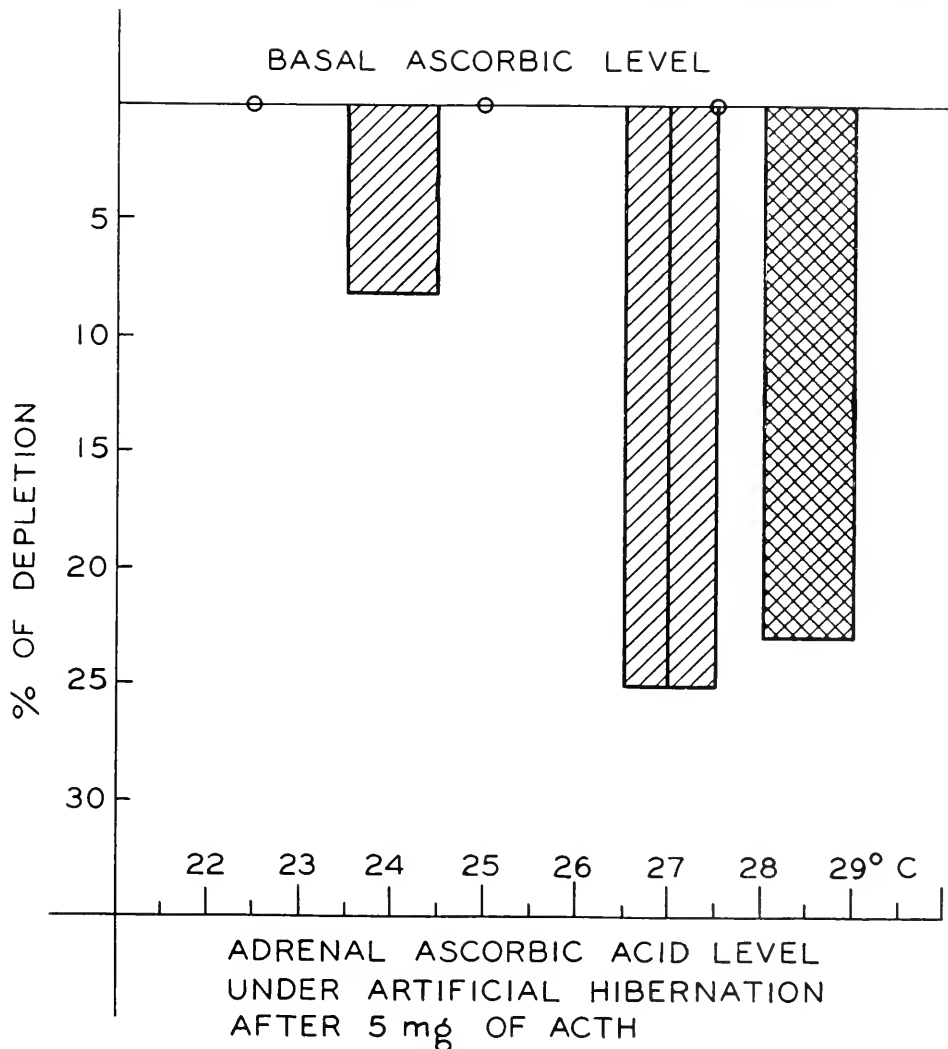


FIG. 1.

milligrams of ACTH. It is interesting that the fall in the ascorbic acid of the adrenals is greater between 29° and 26° C. than between 25° and 23° C. That means that if we want to suppress the reaction of the adrenal to ACTH, it is necessary to decrease the body temperature below 25° C.

The problem is, what is the reaction of the pituitary-adrenal coupled during stress and hypothermic conditions? These results (fig. 2) are applicable to all methods of hypothermia (artificial hibernation, surface cooling), regardless of which drugs are employed to produce hypothermia. These results are also applicable to any stress (formalin injection, or ligation of the hepatic artery or of the mesenteric vein and artery). A stress between 37° and 33° C. is able to depress the ascorbic acid level of the adrenal about 35 per cent, between 32° and 28° C. the fall in the adrenal level is only about 10 per cent, and between 27° and 26° C. there is no fall in the adrenal ascorbic level.

From experiments on more than 1,000 rats we can conclude that there is a critical temperature at which there exists an inhibition of the pituitary reaction. For this reason we thought that between 28° and 26° C. there exists a physiological state which can be compared with an hypophysectomy.

When we compare surface cooling and artificial hibernation, we see that in surface cooling the drop in the adrenal ascorbic level is 28 per cent. When adrenalin or ACTH are injected (I.P.), another fall of about 4.6 to 9.9 per cent is obtained. In fact, when we compare with the controls we see that the same quantity of ACTH is able to depress the adrenal to about 33 to 36 per cent. This suggests that if the adrenals in surface cooling are not able to react more, it is because they are partly exhausted by the cooling.

Dr. Henry Swan: We have been interested in attempting to study hypothermia

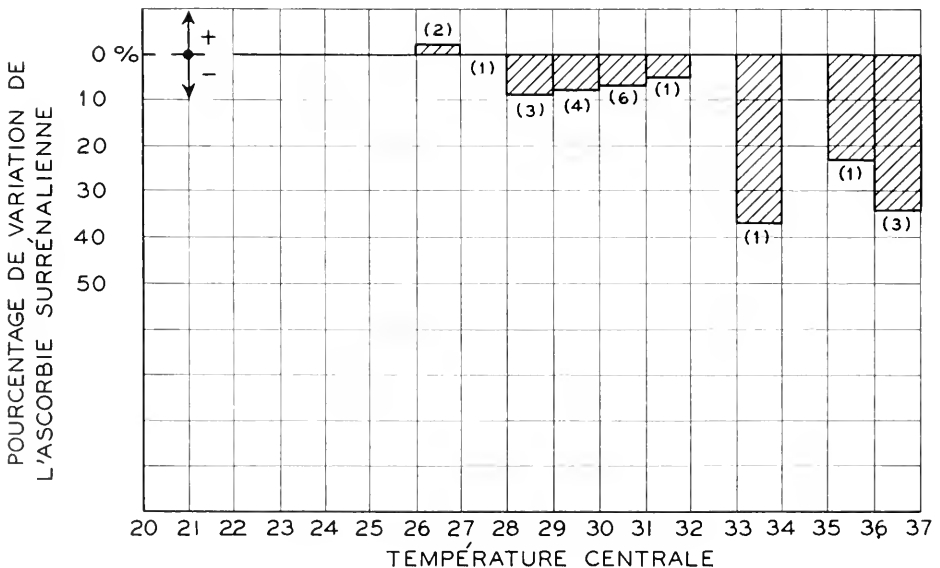


FIG. 2.

in the *unstressed* individual, both human and dog, and we, too, so far have been unable to study the effect of hypothermia in the unstressed individual.

Dr. Humie was studying an animal essentially maximally stressed, and Dr. Bernhard's chart showed that in his experimental animals after the placement of the cannulas, ACTH failed to produce any considerable additional rise in the corticoid output, so when hypothermia was superimposed the animal being studied was in an already stressed state. For that reason it is difficult to estimate the effect of hypothermia on the corticoid output when it is already high.

During a state of hypothermia it is not surprising that the cellular function is apparently markedly depressed as is the cellular function of all the tissues of the body when the cells are quite cold. So during the period of hypothermia this depression of corticoid output is not surprising, but the response afterwards appears to us to be the crux.

We would like to be able to study the effect of hypothermia on an unstressed animal. The critical consideration here, interestingly enough, is anesthesia. In Dr. Bernhard's study the anesthetic agent used in the clinical series was apparently always ether. We have been interested in the cortical response to different types of anesthesia, and I would like to show you one slide in that regard. These are warm patients.

Figure 1 shows the corticoid response to one hour of anesthesia with pentothal alone (or with cyclopropane) and with pentothal-ether. The corticoid response averages an increase of 11 over the control value in six patients who had one hour of ether anesthesia. On the other hand, pentothal or cyclopropane anesthesia in 14 patients appears to cause little or no response in corticoid output. For this reason we plan now to proceed with the obvious step of inducing hypothermia with the use of this anesthetic agent to see if we can study the response in the nonstressed human.

Figure 2 merely shows the possibilities of response. It would appear from Dr. Bernhard's work that hypothermia delays the corticoid response to operation. We show here the initial rise with anesthesia. Possibly with the proper anesthesia one could obtain a straight, level line. One must then measure the immediate postoperative response in the patient who has been under hypothermia. On the basis of present evidence, Dr. Bernhard suggests that it falls in a mid-position, i.e., hypothermia has not completely suppressed the adrenocortical response to surgery, but it has both delayed and diminished it. It would be interesting to see if in the unstressed animal hypothermia can suppress the cortico-corticoid response to trauma.

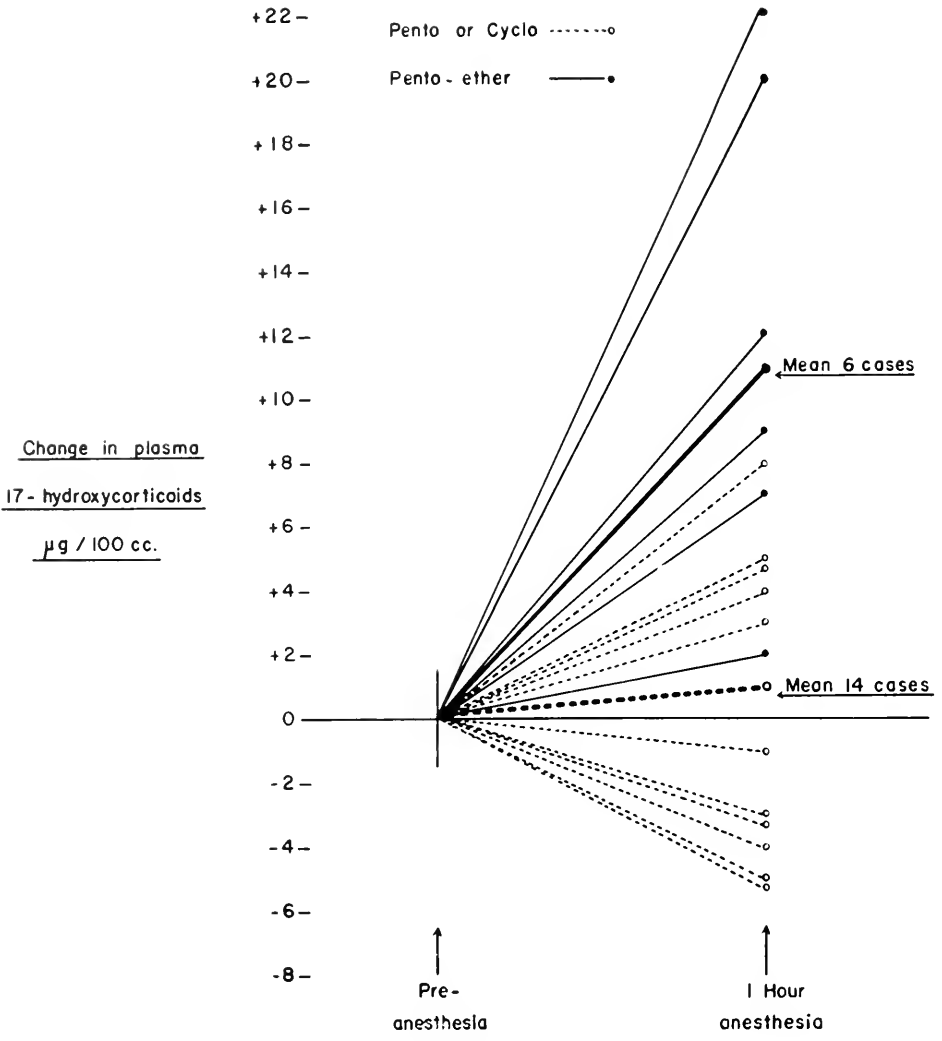


FIG. 1.

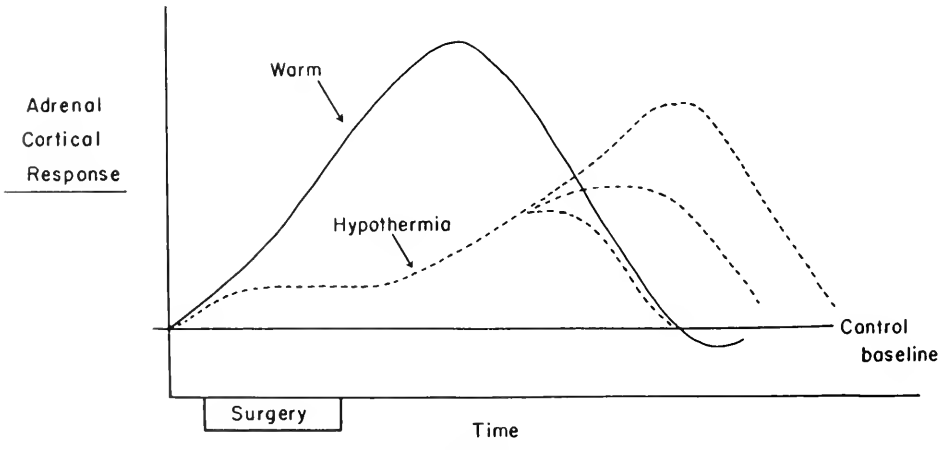


FIG. 2.

SOME PROBLEMS OF HEMATOLOGY IN HYPOTHERMIA: AN INTRODUCTION

WILLIAM H. CROSBY, JR.

About a year ago Colonel Hughes, of the Surgical Division of the Walter Reed Army Institute of Research, reported that his dogs under deep hypothermia tended to bleed, not massively, because the circulation was sluggish, but persistently. This phenomenon was investigated in a collaborative study by the Departments of Hematology and Experimental Surgery.

Figure 1 shows some of the hematologic results which occurred in animals,

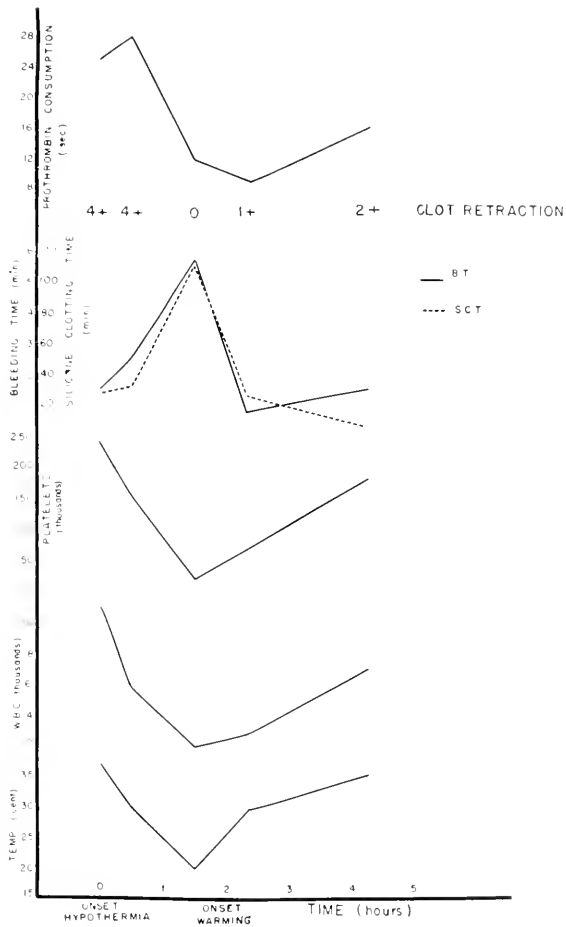


FIG. 1.—This figure correlates the change in white cell, platelets, silicone clotting time, bleeding time, clot retraction and prothrombin consumption with body temperature. It can be seen that as the platelets drop all the various platelet functions are interfered with. This figure also shows the hyper-coagulability in the 2½ hour test of bleeding time and in the 4½ hour test of silicone clotting time (Dog No. 4).

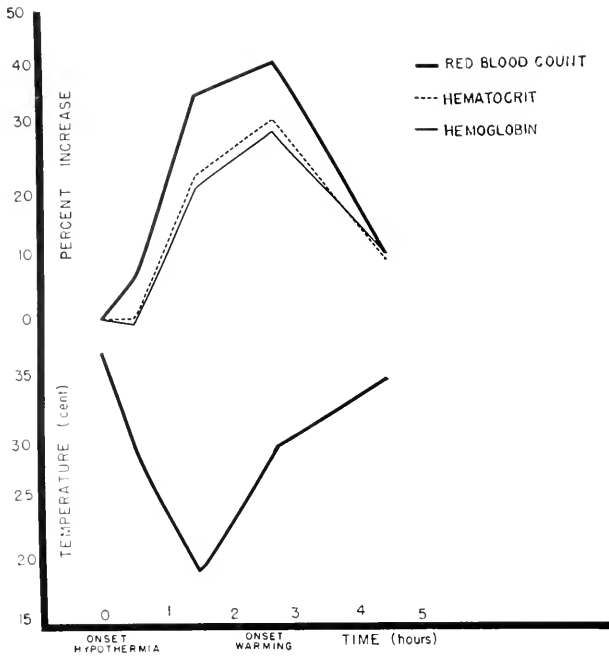


FIG. 2.—Correlating changes in red count, hematocrit and hemoglobin with body temperature. This figure shows the rise in red cell values with cooling and the return to normal with re-warming (Dog No. 4).

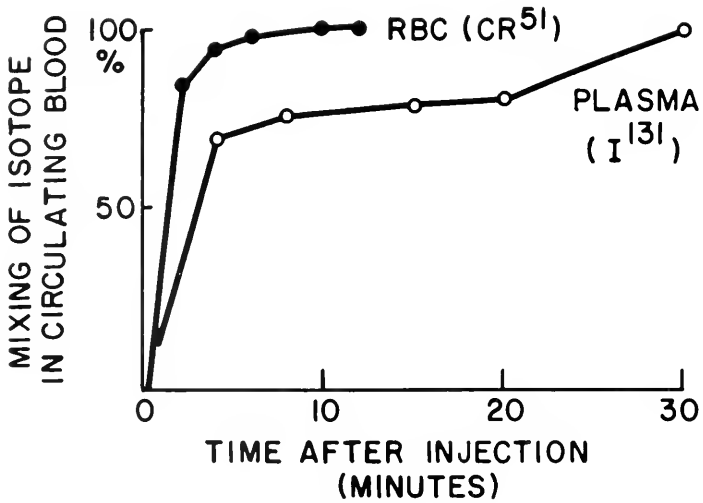


FIG. 3.

surface-cooled to body temperatures of approximately 20° C. The platelets fell to quite low levels, 6,000 to 60,000, as the animal was cooled, and then recovered as it was warmed. The changes in the coagulation mechanism could be accounted for on the basis of thrombocytopenia: the alterations of bleeding time, clotting time, prothrombin consumption, and so forth. The leukocytes also disappeared and then reappeared.

This left us with several problems. What happened to the platelets and leukocytes? When the platelets and leukocyte counts returned to normal, were these the same cells which had been in the blood before hypothermia, or was this a new population?

Figure 2 shows another change which occurred, an increase in hematocrit in most of the animals which recovered rather more slowly than the changes in platelets and leukocytes.

A preliminary experiment (fig. 3) by Dr. W. A. O'Brien seemed to indicate that the change in hematocrit was due to a temporary loss of plasma from the circulating blood.

With this as an introduction, I would like to present Dr. Adelson, who will discuss the changes which take place in the platelets and leukocytes during deep hypothermia.

THE EFFECT OF HYPOTHERMIA ON PLATELETS AND WHITE CELLS IN DOGS

TULIO J. VILLALOBOS, EDWARD ADELSON AND PHILIP RILEY

(With technical assistance of HAROLD GLAUCKE)

In a previous report from this laboratory, results of hematologic studies in nine hypothermic dogs cooled below 20° C. were presented. These results showed a rise in red cell count, hemoglobin concentration and hematocrit; a marked drop in white cell count; and nearly complete disappearance of platelets. On rewarming these changes all returned to normal. A number of studies have been carried out to learn the mechanism of these alterations. In the present paper, we will summarize these studies—especially as they concern the platelets and white cells.

The platelets and white cells, which nearly completely disappear during hypothermia, return within a period of minutes during the early phase of rewarming. The first question which presented itself was whether these returned platelets and white cells are newly formed, or are simply the old platelets and white cells returning to the circulation after a period of sequestration.

To study this question, we applied to animals a technique described by Desai *et al.* in humans. By this technique we injected 2 millicuries of P³² into a normal dog. One week later we withdrew 500 cc. of blood from a vein of this animal. The blood was obtained in plastic bags with sequestrene as anticoagulant. Simultaneously, a second dog which had not received P³² was phlebotomized, and immediately thereafter the second dog received a transfusion of the 500 cc. of whole blood obtained from the radioactive donor dog. This whole blood contained many radioactive substances, among which were radioactive platelets. One-half hour after the infusion, an aliquot of blood was drawn from the recipient animal, the platelets separated, washed and counted. There were 3.7 (10⁻⁹) counts per second per platelet. The animal was then subjected to hypothermia, his platelets nearly disappeared, and the animal was then rewarmed. With the return of his platelets, the radioactivity of aliquots of his platelets also returned. This radioactivity was determined to be 3.0 (10⁻⁹) counts per second per platelet. In other words, 81 per cent of the tagged platelets, which had been infused early in the morning prior to hypothermia, were still present in the afternoon after hypothermia. The slight drop in platelets could easily be explained by the normal life span of the platelet. The fact that 81 per cent of the transfused radioactive platelets were still present showed clearly that the drop in platelets due to hypothermia is a result of sequestration of platelets, not of destruction of platelets. The return of the platelet count toward normal after hypothermia is not due to the production of new platelets, but to the return of the sequestered platelets to the circulation. Figure 1 summarizes these findings.

The next question which presented itself is: where does the sequestration occur? Table I shows that the sequestration does not occur in the capillaries of the body, at least not in the capillaries of the tongue. Here the results of platelet counts in the aorta are compared with those found in the capillary blood in the tongue. It

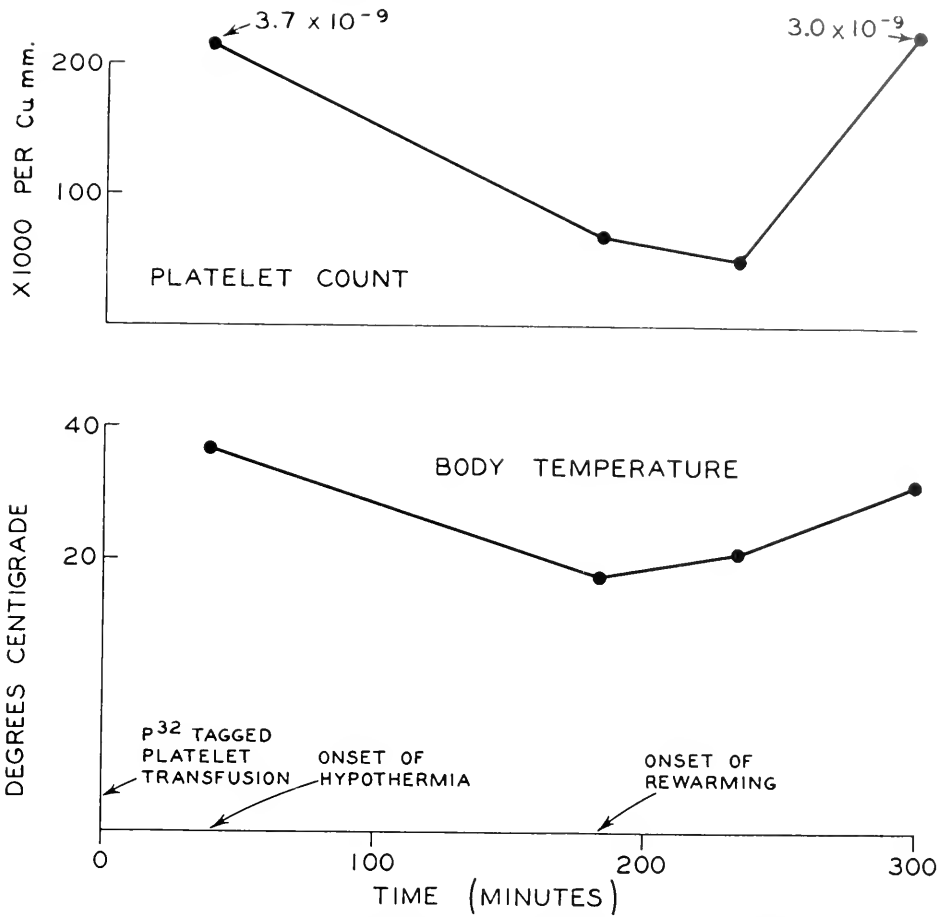


FIG. 1.—Platelet radioactivity P³² (counts per second per platelet).

TABLE I

Temp. ° C.	Platelet counts (per cu. mm.) aorta	Platelet counts (per cu. mm.) capillary blood (tongue)
37.....	445,000	339,000
25.....	213,000	199,000
20.....	28,500	33,000
25.....	75,000	71,500
30.....	451,000	466,000

can be seen that there is no significant difference. Table II shows that capillaries of the spleen, liver, and lung also do not sequester platelets. Tables III, IV, and V show the results of platelet counts carried out simultaneously in the aorta and high in the inferior vena cava in three animals. These counts were taken by means of polyethylene catheters, positioned by means of incisions in the femoral artery and femoral vein. The catheter high in the inferior vena cava was positioned well above the entrance of the hepatic vein into the inferior vena cava. Therefore, it sampled a

TABLE II

Dog no.	Platelet counts (per cu. mm.) at maximum hypothermia			
	Aorta	Spleen	Liver	Lung
11.....	28,500	31,500	10,000	6,000
12.....	24,000	55,000	13,500	10,000
13.....	68,500	59,500	20,500	11,500
18.....	41,000	38,500	25,500	20,000

TABLE III

Temp. ° C.	Platelet counts (per cu. mm.)	High inferior vena cava platelet counts (per cu. mm.)
37.....	286,000	230,000
25.....	92,500	37,500
20.....	45,500	16,000
25.....	27,500	94,500
30.....	77,500	118,000

TABLE IV

Temp. ° C.	Aorta blood		High inferior vena cava	
	Platelet count (per cu. mm.)	WBC (per cu. mm.)	Platelet count (per cu. mm.)	WBC (per cu. mm.)
37.....	365,500	6,500	316,500	6,400
25.....	102,500	2,300	66,500	3,800
20.....	24,500	1,800	20,500	1,500
25.....	131,500	2,900	187,500	2,800
30.....	186,000	3,400	197,500	2,850

TABLE V

Temp. ° C.	Aorta blood			High inferior vena cava		
	Platelet count (per cu. mm.)	Hgb	WBC (per cu. mm.)	Platelet count (per cu. mm.)	Hgb	WBC (per cu. mm.)
37.....	332,000	13.6	14,800	298,000	13.3	15,950
25.....	183,000	16.2	10,900	131,000	15.7	13,250
20.....	68,500	19.3	6,250	35,000	18.7	6,500
25.....	144,500	14.7	8,800	195,000	12.7	9,850
30.....	220,000	12.4	10,850	249,000	13.1	11,850

mixture of blood from both the systemic and portal circulations. The results show that the drop in platelet count high in the inferior vena cava preceded the drop in platelets in the aorta. During rewarming the rise in platelets high in the inferior vena cava also preceded that of the platelet rise in the aorta. In other words, it seemed that the platelets were sequestered and later released from a site somewhere between the aorta and the high inferior vena cava catheters. In tables VI and VII we see the results obtained when the venous catheter was placed low in the inferior vena cava—inferior to the entrance of the hepatic vein to the inferior vena cava. In these two dogs it could be seen that the inferior vena cava blood no longer preceded the aorta blood in the drop of platelets. This seems to indicate that the site of platelet sequestration and subsequent platelet release is located in the portal, rather than in the systemic circulation. In table VIII platelet counts are compared in the

TABLE VI

Temp., ° C.	Aorta blood		Low inferior vena cava	
	Platelets (per cu. mm.)	WBC (per cu. mm.)	Platelets (per cu. mm.)	WBC (per cu. mm.)
37.....	177,000	8,900	218,000	9,700
25.....	81,000	3,200	90,500	3,600
20.....	20,000	1,900	30,000	1,800

TABLE VII

Temp., ° C.	Aorta blood		Low inferior vena cava	
	Platelets (per cu. mm.)	WBC (per cu. mm.)	Platelets (per cu. mm.)	WBC (per cu. mm.)
37.....	299,500	15,000	342,500	13,000
25.....	123,000	8,600	117,500	9,900
20.....	48,500	8,000	47,500	8,800

TABLE VIII

		Temp., ° C.			
		37	25	20	25
Platelets (per cu. mm.)	Aorta	316,000	61,500	41,000	28,000
	Low inferior vena cava...	356,000	88,500	42,000	18,000
	Hepatic vein	247,000	26,500	27,500	—
White blood cells (per cu. mm.)	Aorta	13,200	6,600	4,900	9,000
	Low inferior vena cava...	12,200	6,700	4,200	5,700
	Hepatic vein	6,800	4,300	4,500	—

TABLE IX

		Temp., ° C.				
		37	25	20	25	30
Platelets (per cu. mm.)	Aorta	374,000	91,000	48,000	177,000	163,500
	Low inf. vena cava..	336,000	108,000	42,500	199,500	226,500
	Hepatic vein	363,000	74,500	30,000	201,000	189,000
White blood cells (per cu. mm.)	Aorta	7,000	2,600	1,400	2,300	3,800
	Low inf. vena cava..	5,900	3,000	1,800	3,700	5,000
	Hepatic vein	6,000	2,000	1,200	3,900	5,500

aorta, low inferior vena cava, and hepatic vein. Here it can be seen that the hepatic vein leads the other samples in the platelet drop. This is further proof that the site of sequestration lies somewhere in the portal circulation. In table IX, a similar result is seen. In addition, this shows the hepatic vein leading the other samples in the platelet rise. In table X the results of platelet counts in the aorta, low inferior vena cava, portal vein just inferior to the liver, and hepatic vein are compared. In this animal, the portal vein and hepatic vein samples both lead the platelet drop and the platelet rise. This would seem to indicate that the liver cannot be the *only* site of platelet sequestration. However, the fact that the hepatic vein counts are lower on the way down and higher on the way up than the portal vein platelet counts seems to indicate that the liver does play a considerable role in these changes. In tables XI and XII are shown the results of splenectomy on the hematologic changes in hypothermia. In neither animal was the platelet drop or white cell drop signifi-

TABLE X

		Temp. ° C.				
		37	25	20	25	30
Platelets (per cu. mm.)	Aorta	242,000	106,000	27,000	64,000	129,500
	Low inf. vena cava.	233,000	82,000	23,000	73,000	126,000
	Portal vein	212,000	53,500	21,000	77,500	141,500
	Hepatic vein	146,500	40,000	11,500	101,000	150,000
White blood cells (per cu. mm.)	Aorta	18,400	6,300	4,000	6,000	7,700
	Low inf. vena cava..	18,200	8,400	—	5,300	7,900
	Portal vein	11,600	6,000	3,800	4,000	8,300
	Hepatic vein	11,200	8,300	4,100	5,900	7,900

TABLE XI

HYPOTHERMIA IN SPLENECTOMIZED DOG

Temp. ° C.	Aorta blood			High inferior vena cava		
	Platelets (per cu. mm.)	Hemoglobin	WBC (per cu. mm.)	Platelets (per cu. mm.)	Hemoglobin	WBC (per cu. mm.)
37.....	360,500	16.8	11,400	257,000	17.6	9,100
25.....	149,000	18.7	7,800	115,000	18.0	6,300
20.....	58,500	18.0	6,000	49,000	18.3	5,800
25.....	98,500	16.4	3,000	135,000	16.8	5,000
30.....	145,500	17.8	5,500	205,500	17.3	5,900

TABLE XII

HYPOTHERMIA IN SPLENECTOMIZED DOG

Temp. ° C.	Aorta blood			High inferior vena cava		
	Platelets (per cu. mm.)	Hemoglobin	WBC (per cu. mm.)	Platelets (per cu. mm.)	Hemoglobin	WBC (per cu. mm.)
37.....	369,500	14.3	24,900	262,000	13.8	20,500
25.....	138,000	15.7	8,200	108,000	15.1	6,100
20.....	42,500	16.4	5,200	34,000	15.2	6,100
25.....	75,500	15.1	6,600	95,000	15.5	7,000
30.....	198,500	16.2	12,600	167,000	15.9	12,000

TABLE XIII

TOTAL HEPATECTOMY—COUNTS FROM AORTA

Temp. ° C.	Platelets (per cu. mm.)	WBC (per cu. mm.)	HCT
37.....	237,000	11,200	43.5
25.....	168,000	10,900	40.7
20.....	138,000	8,700	38.7
35.....	144,000	9,400	39.4
30.....	265,000	15,000	40.1

cantly altered, nor was the hemoglobin rise changed. This would seem to indicate that the spleen alone cannot be a major factor. In tables XIII and XIV the results of total hepatectomy on the platelet drop of hypothermia are studied. The hepatectomy appears to have decreased the platelet drop, the white cell drop and the hematocrit rise. However, in table XIV hepatectomy does not appear to have had a significant effect. Incidentally, the major surgical procedure which the animal underwent can easily explain the hematocrit drop seen in table XIII.

TABLE XIV

TOTAL HEPATECTOMY COUNTS FROM AORTA

Temp. ° C.	Platelets (per cu. mm.)	WBC (per cu. mm.)	Hct
37.....	248,000	13,200	53.9
25.....	135,500	11,000	57
20.....	54,000	6,500	55.5
25.....	133,000	7,400	55.5
30.....	131,000	15,900	57.5

TABLE XV

HEPATECTOMY AND SPLENECTOMY—COUNTS FROM AORTA

Temp. ° C.	Platelets (per cu. mm.)	WBC (per cu. mm.)	Hematocrit
37.....	221,000	7,950	53
25.....	137,000	8,700	58
20.....	106,000	6,700	54

Died.

TABLE XVI

HEPATECTOMY AND SPLENECTOMY

Temp. ° C.	Upper inferior vena cava			Lower inferior vena cava		
	Platelets (per cu. mm.)	HCT	WBC (per cu. mm.)	Platelets (per cu. mm.)	HCT	WBC (per cu. mm.)
37.....	268,000	54	6,700	265,000	56	6,350
25.....	211,000	53	6,000	167,000	53	5,000
20.....	103,500	52	3,500	110,500	52	3,650
25.....	104,000	49	5,850	108,500	49	5,200
30.....	143,500	52	7,550	199,500	52	10,800

TABLE XVII

HEPATECTOMY AND SPLENECTOMY

Temp. ° C.	Upper inferior vena cava			Lower inferior vena cava		
	Platelets (per cu. mm.)	HCT	WBC (per cu. mm.)	Platelets (per cu. mm.)	HCT	WBC (per cu. mm.)
37.....	196,500	53.5	6,750	160,000	54	7,150
25.....	134,500	52.5	3,825	136,000	53	4,180
20.....	96,000	51	3,400	114,000	52	3,580
25.....	Clumped	48.5	3,950	Clumped	50	2,800
30.....	74,000	50	4,600	60,500	50.5	4,300

In tables XV, XVI, XVII, and XVIII the effect of combination hepatectomy and splenectomy are studied. In all four animals the platelet and white cell drop appear to be markedly decreased. No definite conclusion can be drawn from the hematocrit changes since we feel that the major surgical procedure was sufficient to alter blood volumes and therefore hematocrits. In tables XVII and XVIII the animals did not show the warming phase rise in platelet count. We feel this was due to the marked trauma the animals had undergone and the resultant extreme cachexia. Figures 2 and 3 summarize the results of platelet and white cell counts in control animals, hepatectomized animals, splenectomized animals, and hepatectomized-splenectomized animals. Splenectomy alone had little effect, hepatectomy alone a slightly greater effect,

TABLE XVIII
HEPATECTOMY AND SPLENECTOMY

Temp. ° C.	Upper inferior vena cava			Lower inferior vena cava		
	Platelets (per cu. mm.)	HCT	WBC (per cu. mm.)	Platelets (per cu. mm.)	HCT	WBC (per cu. mm.)
35.....	333,000	60	7,200	357,500	58.2	6,700
25.....	264,000	57.9	6,350	203,500	56.8	5,500
20.....	181,000	57	5,450	154,500	55.2	4,700
27.....	170,000	57	3,400	162,000	56.5	3,300
35..... (terminal)	102,000	55	2,800	101,000	55.8	3,300

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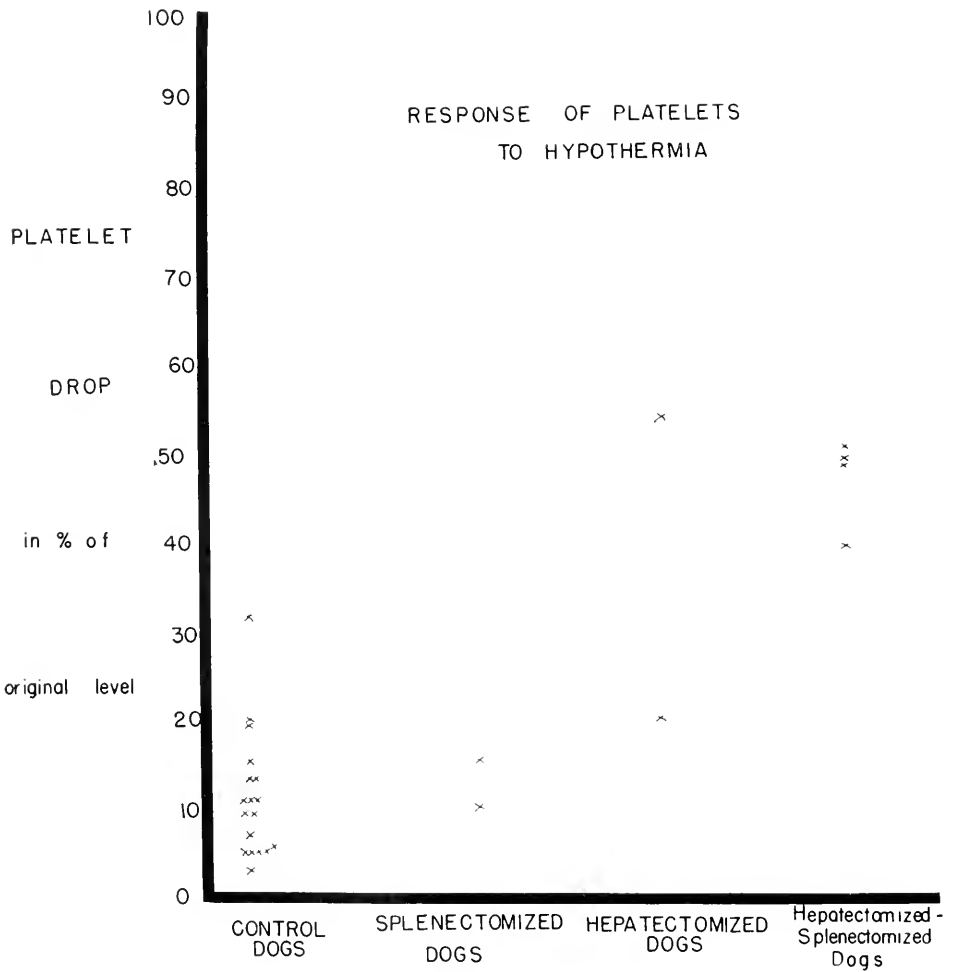


FIG. 2.

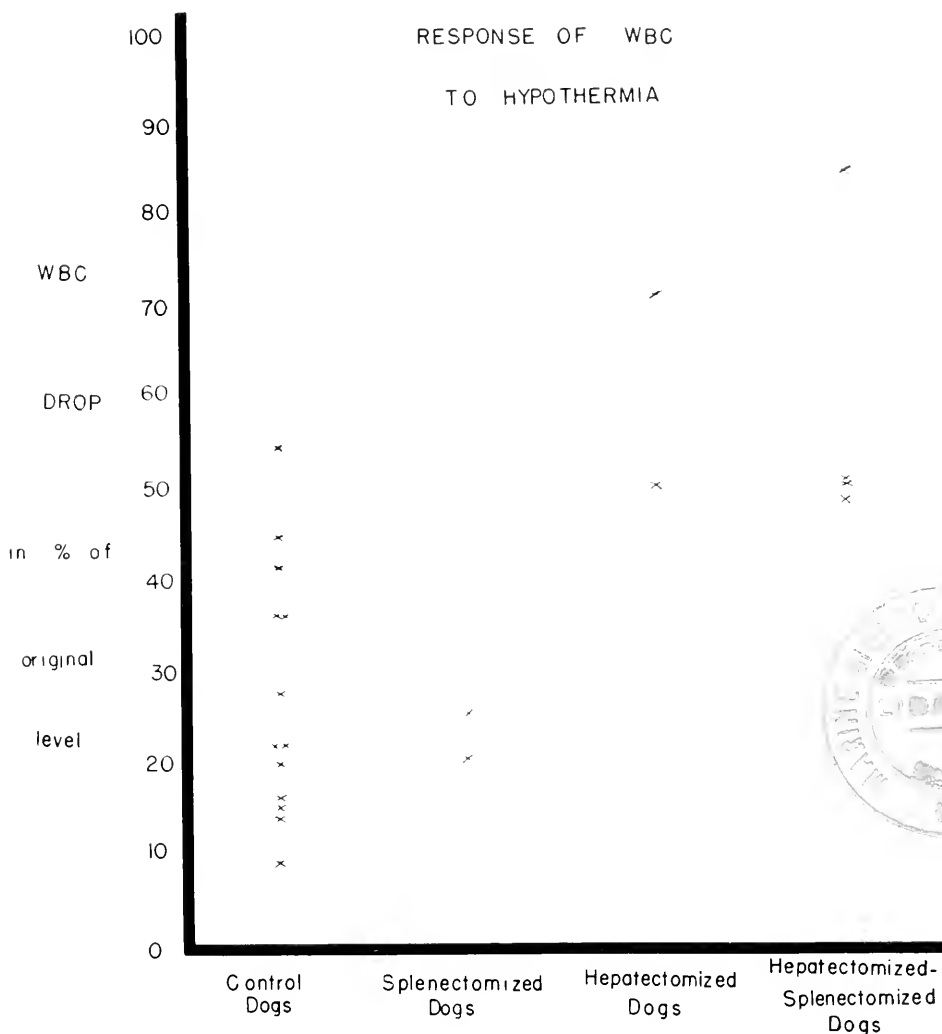


FIG. 3.

and the two procedures together reduced the lowering of platelets considerably. Similar results, although not quite as clear-cut, were obtained with white cell counts.

Summary. The decrease in platelet count and probably white cell count in hypothermic dogs is due to the sequestration of platelets and white cells, and not to their destruction. Catheterization studies indicate that some of the sequestration occurs in the liver and probably also in the spleen. However, since hepatectomy and splenectomy did not completely abolish the platelet and white cell drops, we feel that other sinusoidal organs such as the bone marrow may also play a role in the sequestration of platelets and white cells.

DISCUSSION

Dr. C. M. Courtes: We, too, have been interested in the hematologic changes resulting from induced hypothermia. Prior to our study, it was our clinical impression that hypothermic dogs seemed to have an increased oozing tendency but that blood shed during surgery into the peritoneal or pleural cavity seemed to clot adequately. We undertook to investigate the oozing tendency. Altogether, 62 dogs were cooled to a depth of from 18°–25° C. for from one to four hours, and rewarmed with a Therm-O-Rite machine. For most of the studies arterial blood, obtained from the femoral artery, was used for the determinations.

Our studies included clotting times in glass (Lee White), bleeding times, clot retraction, hemoglobin estimation, hematocrit estimation, platelet count, prothrombin times in plasma (one stage), prothrombin times in serum, mechanical fragility, osmotic fragility, critical fibrinogen index, heparin activity and smears of peripheral blood. Results are summarized in figures 1, 2, 3.

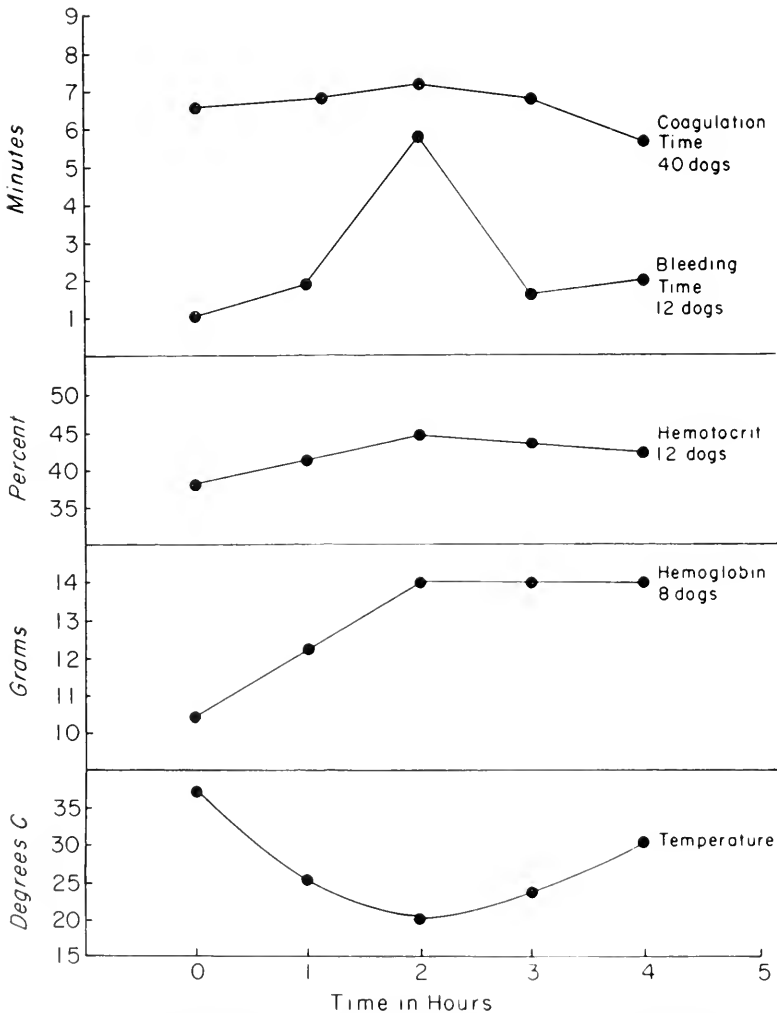


FIG. 1.—Coagulation time, bleeding time, hematocrit, hemoglobin.

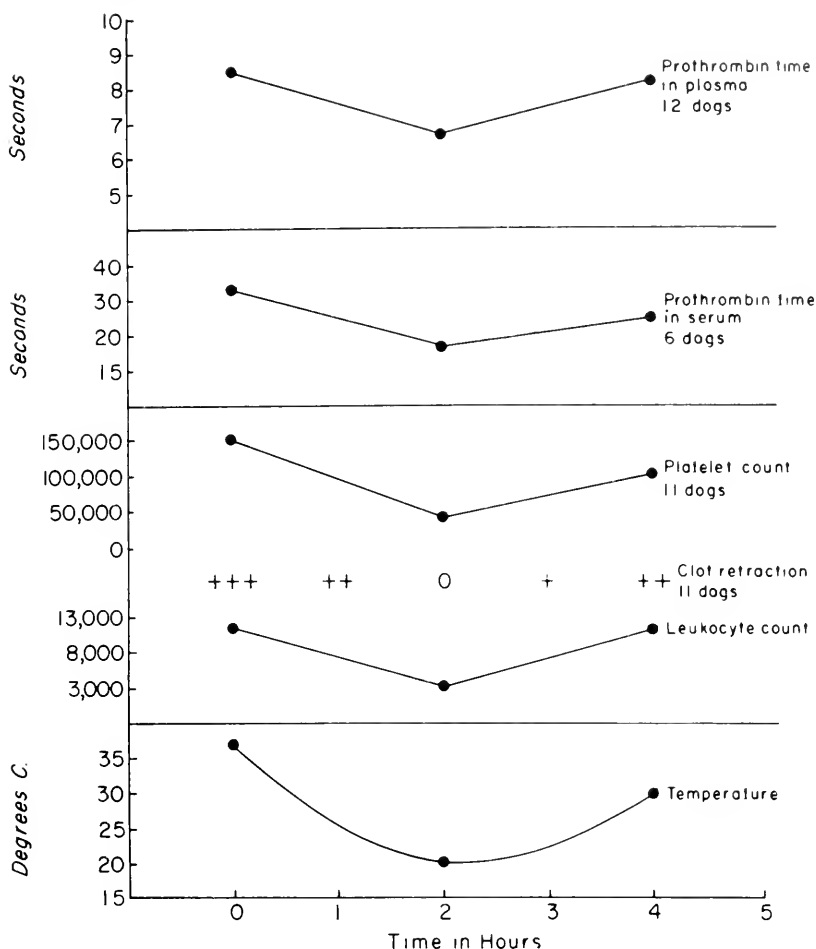


FIG. 2.—Prothrombin time in plasma, prothrombin time in serum platelets, clot retraction, leukocyte count.

Coagulation times in glass were not significantly altered by cooling. Coagulation times in silicone tubes were altered as others have shown. This difference is no doubt due to the reduction in the platelets which occur with cooling.

There was a five-fold increase in the bleeding time as a result of cooling. Five of the 12 dogs tested had a seven-fold increase at reduced temperatures. One dog had a bleeding time of 17 minutes, which falls well beyond the normal range. This process is completely reversible with rewarming.

The hemoglobin and hematocrit invariably rise with cooling.

The prothrombin time in plasma by the one stage method was not greatly altered by cooling in our series. However, the prothrombin time in serum is significantly decreased. This being a measure of residual prothrombin, it is quite possible that this decrease could be due to the platelet drop which occurs.

The platelet count drops to a very low level with cooling. In some of our animals

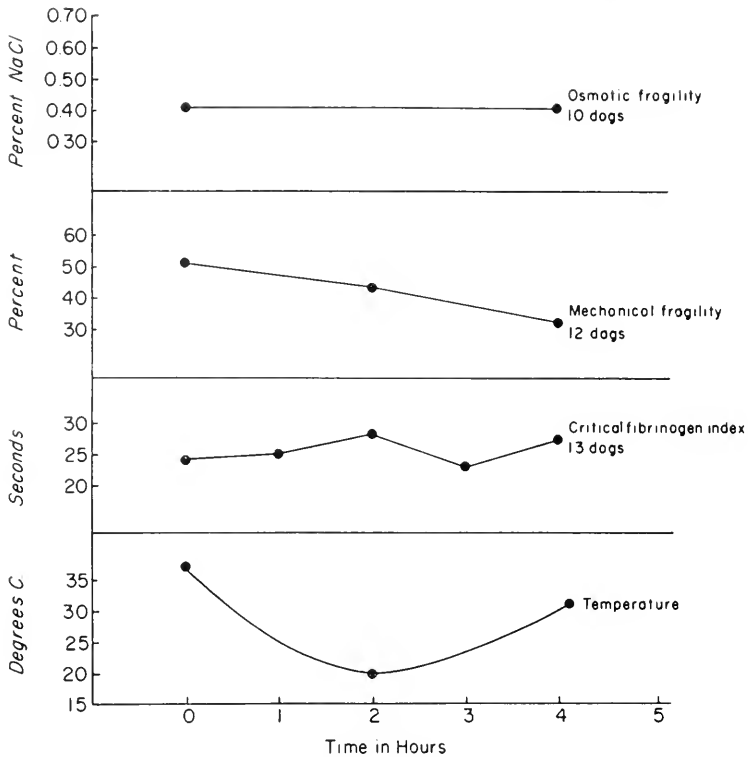


FIG. 3.—Osmotic fragility, mechanical fragility, critical fibrinogen index.

at 20° C. it was common to find only 10,000 platelets. These are not destroyed, for they return with rewarming.

Along with drop in the platelet count, the clot retraction is poor.

There is a marked drop in leukocytes at reduced temperatures. The mean at the reduced level was 3047. They return to normal with rewarming.

Heparin activity, osmotic and mechanical fragility, and the critical fibrinogen index are not affected by cooling.

There is a relative increase in the number of lymphocytes with cooling.

Dr. Dean Warren: We can confirm the constant decrease in white blood counts and platelets, the rise in hemoglobin and hematocrit, and the prolongation of coagulation time. We, too, thought that sequestration was probably the phenomenon responsible for this, but were not able to localize it accurately through our more crude method of splenectomy.

One factor that we thought might be of importance was that of the delayed adrenocortical response. As has been shown by Ebert, cortisone is somewhat effective in preventing this sequestration phenomenon in other experimental conditions. We gave large doses of cortisone to dogs for 48 hours prior to our experiments, but the fall was similar in all phases of our experiment. Heparin was likewise ineffective in changing this response.

In addition, we have studied two patients. Although the temperatures were not

carried to the low levels reached in dogs, both patients showed the preliminary decreases found at comparable temperatures in the animals.

Dr. Jonathan Rhoads: If the liver and spleen do not completely account for sequestration of platelets and white cells, could the gut be a reservoir?

Dr. Adelson: We did not study this. I would like to point out that coagulation-time studies in glass tubes are less accurate than when carried out in silicone tubes.

Dr. James A. Helmsworth: The hematologic aspects of hypothermia attracted our attention because of the development of an hemorrhagic diathesis in dogs surviving cooling by immersion in ice water in our laboratory. A large proportion of these animals had profuse bleeding into the intestinal tract and died in shock within 18 hours after chilling. Our findings in reference to the changes in the platelet count confirm those of Crosby and Adelson. The average reduction in platelet count was 86% in dogs cooled by this method to a body temperature of approximately 25° C. The same measurements were carried out in a small group of monkeys cooled by the same method and to the same extent, and in these animals there was the same marked thrombocytopenic effect. There are two additional comments which are of some interest. The first is that, in dogs cooled by the same method and to the same temperature, the thrombocytopenic effect was much less if autonomic blockade had been established by Arfonad. Indeed, in some of these "blocked" animals the platelet count remained unchanged. The second is that dogs cooled by means of a simple extracorporeal circuit do not show the same fall in platelet count. In this group of animals with blood flowing from femoral artery to femoral vein through a simple plastic tube the platelet count has, on many occasions, remained normal even though the changes in body temperature were of the same degree mentioned before.

Dr. J. Adams-Ray: We have made some observations that seem to fit with the sequestration of blood in the liver in hypothermia as indicated by Dr. Adelson. The method used for measuring variations of the liver volume is a roentgenstereophotogrammetrical one.¹ Small silver pellets are placed on the liver so that they enclose a polyhedron. With two X-ray tubes with known distance in relation to film these will be photographed at intervals during the experiment. Each pellet will then be reproduced at two places. Measuring the distance between the two images of each pellet, and knowing the distance between the X-ray tubes and the distance between the tubes and film, we can locate each pellet in an orthogonal-coordinate system. The volume of the polyhedron enclosed by the pellets can then be computed and the mean error of the volume determinations is somewhat below 1 per cent of the total volume measured. The calculations are easily made by using a punch-card system in a calculating machine.

In hypothermia we found a slight increase in liver volume,² observable even at 26° to 28° C. rectal temperature. This was in contrast with the considerable decrease of liver volume that my associate, Dr. Hagberg, found in hemorrhagic shock, which would give anoxia, possibly interfering *inter alia* with the bacterial defence mechanisms described by Jacob Fine. When Dr. Hagberg induced hypothermia during such experiments the decrease of liver volume was inhibited. His experiments are not yet concluded but indicate a protecting effect of hypothermia

just as Friedman, Frank and Overton have found; the effect seems to be coupled to the inhibition of liver volume decrease in hemorrhagic shock.

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RENAL FUNCTIONAL RESPONSE TO HYPOTHERMIA AND ISCHEMIA IN MAN AND DOG

JOHN H. MOYER, GEORGE C. MORRIS AND MICHAEL E. DEBAKEY

With the use of homograft replacement of the abdominal aorta, interruption of the blood supply to the kidney frequently becomes necessary. If the renal ischemia is prolonged, irreversible renal damage may result. Therefore, it becomes necessary to explore methods for either maintaining the blood supply to the kidney or reducing the metabolic processes within the kidneys so that irreversible renal damage does not result from the ischemia. One approach would be the use of hypothermia to reduce the metabolic requirement of these tissues. Investigation of this problem was undertaken in the following study. The observations can be divided into three parts: (I) observations on the effect of hypothermia on the kidney; (II) observations on the effect of ischemia on the kidney and the resultant renal damage produced by this ischemia and; (III) the protective effect of hypothermia against the renal damage produced by ischemia.

METHODS

The effect of hypothermia on renal hemodynamics and on water and electrolyte excretion. The effect of hypothermia was studied in 20 dogs. The dogs were anesthetized with pentobarbital (30 mg/kg). Following adequate control observations, the animals were made hypothermic with a fluid cooled blanket and an electrically controlled temperature regulator (Therm-O-Rite Product). The body temperature was slowly reduced to 80° F. over a one to two hour period, maintained at this level for two hours, and then slowly increased to the control levels.

Observations were made on glomerular filtration rate and renal plasma flow using creatinine and para-aminohippurate clearances respectively. Sodium and water excretion were determined using the Beckman Flame Photometric method. After the dogs were anesthetized, three 10-minute control periods were collected. As the temperature was lowered, two 10-minute periods were collected at 32° C. (90° F.) and three periods at 26° C. (80° F.). After the temperature had been maintained at 25 to 27° C. for two hours, three additional 10-minute periods were collected. Then the temperature was increased to the control levels and observations on renal hemodynamics were again made for three 10-minute periods. These were repeated 24 hours later. Analytical methods and techniques employed have been described previously.¹

After it was observed that glomerular filtration rate and renal blood flow were depressed during the hypothermic period, an attempt was made to rule out the blood pressure component by elevating the pressure back to the control levels with vasopressor agents (norepinephrine or Aramine). It was reasoned that returning blood pressure to the control levels would help to rule out the effect of hypotension on glomerular filtration rate and renal blood flow.

The effect of renal ischemia on renal hemodynamics and water and elec-

trolyte excretion. In this study 21 dogs (group 2) were used. They were subdivided into three subgroups of seven each. Following suitable control observations on water and electrolyte excretion, and on glomerular filtration rate and renal blood flow, the blood flow to the kidney was interrupted by one of three methods. In the animals in subgroup 2A (fig. 1) the aorta was occluded just proximal to the renal arteries for two hours. Therefore, any blood that circulated through the kidneys following this procedure was due to collateral circulation and retrograde flow into the aorta below the renal arteries. In subgroup 2B, the left renal artery was occluded with a bulldog vascular clamp for two hours without occluding the aorta.

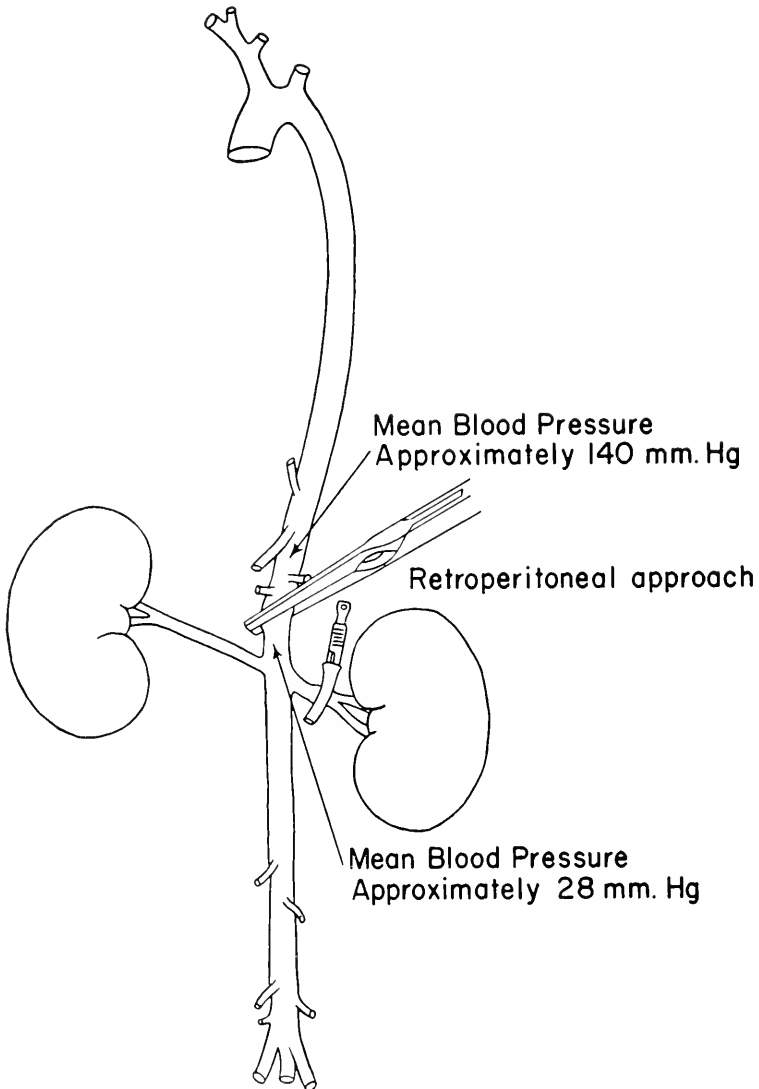


FIG. 1.—Diagrammatic representation of abdominal aorta and renal arteries in the dog showing the points at which the aorta and the left renal artery were occluded.

In the animals in subgroup 2C, the aorta was occluded above the renal artery for two hours with a Potts clamp and at the same time, the left renal artery was occluded with the bulldog clamp. During this procedure, then, the right renal artery was open to any retrograde flow that might occur through the collateral circulation into the aorta below the Potts clamp, but the left kidney was completely blocked of any circulation from the aorta. Following occlusion of the aorta above the renal arteries (subgroups 2B and 2C), the blood pressure distal to the occlusion ranged between 15 and 34 mm. Hg (average 28 mm. Hg), due to the collateral circulation into the distal aorta. The renal artery and the aorta were occluded for two hours in each instance (subgroups 2B and 2C). After the occlusion, the animals were allowed to recover and were placed in their cages. Three days later, the animals were again anesthetized with 30 mg/kg of pentobarbital. Each ureter was then catheterized and observations on renal function were again repeated. At the same time water and electrolyte excretion studies were carried out. This allowed us to determine renal blood flow, glomerular filtration rate, and water and electrolyte excretion for each kidney separately, and thus to compare the degree of damage between the right and the left kidneys as reflected in the alterations in renal hemodynamics.

The effect of renal ischemia during hypothermia. Seven animals were studied in which the temperature was reduced to 25 to 27° C. Then the aorta was occluded above the renal arteries. At the same time the left renal artery was occluded similar to the animals in group 2C. After two hours of occlusion, the clamps were removed and the dogs were warmed up to control levels. Three days later, the ureters were catheterized and observations were made on renal function for each kidney. These were then compared to the observations made in the animals in which the blood supply to the kidneys had been interrupted (subgroup 2C) under normothermic conditions.

RESULTS

The effects of hypothermia on renal hemodynamics and water and electrolyte excretion. Figure 2 summarizes the effect of progressive hypothermia on renal function. The observations are expressed in per cent of the control observations made prior to reducing the temperature. As the temperature was reduced to approximately 90° F. (30 to 32° C.) the average mean blood pressure decreased to 90 per cent of the control levels. This was associated with a reduction in glomerular filtration rate to 58 per cent of the control values and in renal blood flow to 69 per cent of the control level. There was no effect on the hematocrit in this group of animals. When the temperature was reduced to 80° F., i.e. 25 to 27° C., the average mean blood pressure decreased further to about 75 per cent of the control values and the glomerular filtration rate and renal blood flow to 31 per cent and 28 per cent of the control values respectively. The average absolute values for these observations are recorded within the bar graph for each of the functions studied in figure 2.

Associated with the reduction in glomerular filtration rate, there was no concurrent reduction in urine volume or in sodium excretion (fig. 3). This is of interest since under normothermic conditions if the glomerular filtration rate is

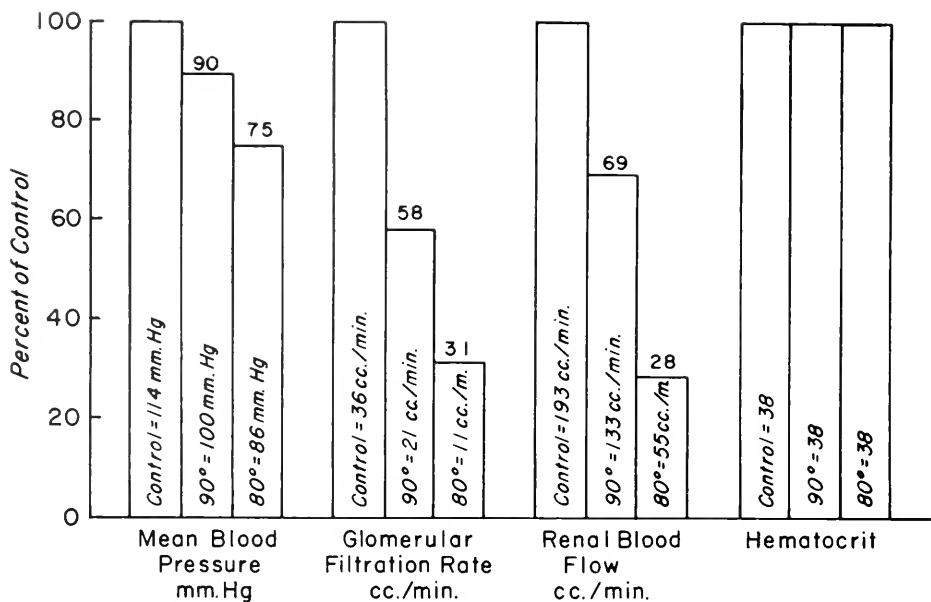


FIG. 2.—Bar graph summarizing the effect of hypothermia on mean blood pressure and renal hemodynamics. The mean values for the group of dogs is enclosed within the bar graph and the per cent change from the control values is indicated at the top of the bar.

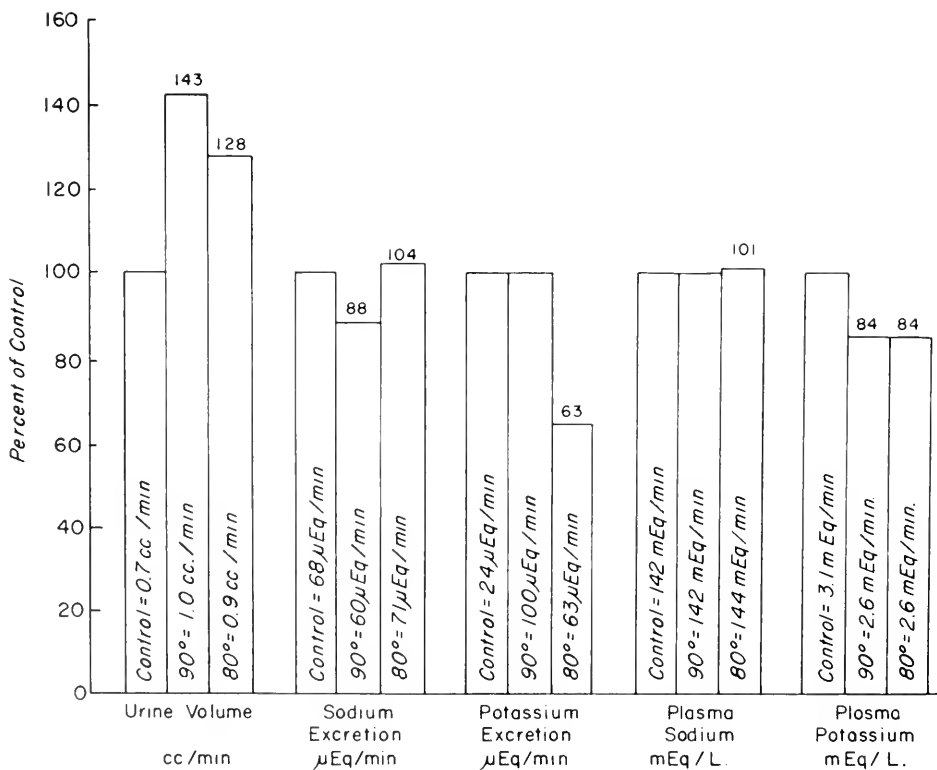


FIG. 3.—Bar graph summarizing average changes of sodium and electrolyte excretion in the same dogs for which renal hemodynamics have been summarized in figure 2.

reduced to this extent (by numerous methods) there is usually a very dramatic reduction in water and sodium excretion. In the current experiments, when the temperature was reduced to 30 to 32° C, there was actually an increase in water excretion to 143 per cent of the control values at the same time that glomerular filtration rate was reduced to 58 per cent of the control values. Observations made at a temperature of 25 to 27° showed little additional effect on sodium and water excretion, although potassium excretion decreased to 63 per cent of the control values.

There was no consistent effect on plasma sodium and potassium. Despite a reduction in glomerular filtration rate and renal blood flow, sodium and water excretion were not reduced, which suggests that hypothermia depresses tubular enzymatic activity and thus reduces the reabsorptive capacity of the renal tubules. The effect on potassium excretion further supports this concept since under normothermic conditions potassium is actively excreted by the renal tubules. The reduction in potassium excretion with a concurrent increase in sodium and water excretion leads to the conclusion that enzymatic processes responsible for reabsorptive and secretory mechanisms are depressed during hypothermia.

The effect of prolonged hypothermia at 25 to 27° C. is summarized in figure 4. It is obvious that prolonged hypothermia had no additional effect on renal hemodynamics. After two hours of hypothermia, the depression in blood pressure, glomerular filtration rate, and renal blood flow was about the same as it was immediately after the reduction in temperature to these levels. These observations indicate that the depression in renal function is a direct response to the hypothermia and the degree of hypothermia rather than to the length of time the animal is kept at reduced temperatures. When the temperature is again raised to control levels there is an immediate increase in blood pressure back to the control levels. How-

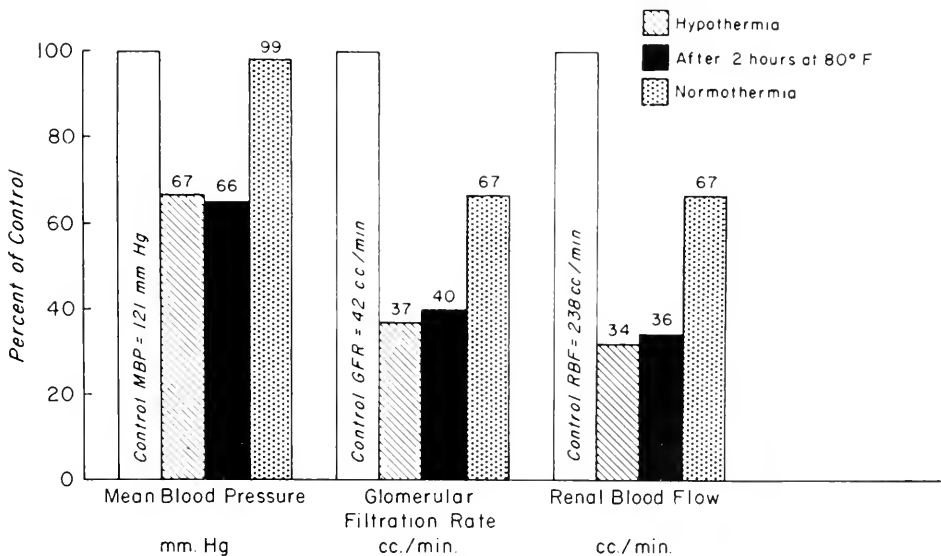


FIG. 4.—The renal hemodynamic effect of hypothermia immediately after reduction in temperature as compared to the responses observed after two hours of maintained hypothermia.

ever, glomerular filtration rate and renal blood flow increase more slowly, being only about $\frac{2}{3}$ of the control levels immediately after normothermic levels are reached. When these observations were repeated 24 hours later, all of the functions had returned approximately to control values.

As with glomerular filtration rate and renal blood flow, prolonged hypothermia had no additional effect on water and electrolyte excretion as compared to observations made immediately after reduction in temperature. There was no change or increase in urine volume and sodium excretion. Potassium excretion was uniformly depressed (fig. 5). In this group of animals it seemed that the effect on water excretion was more pronounced after prolonged hypothermia than it was immediately after the reduction in temperature. When the temperature returned to the control values, sodium excretion remained elevated.

When the blood pressure was raised to control levels with norepinephrine during hypothermia, glomerular filtration rate and renal blood flow were not increased (fig. 6). This suggests that the reduction in renal function is a direct result of hypothermia, not a result of the hypotension. When the temperature was returned to control levels, glomerular filtration rate and renal blood flow both increased.

The effect of renal ischemia on renal hemodynamics and water and electrolyte excretion. In the group of animals in which the aorta was occluded above the renal arteries (subgroup 2A) no alteration in renal function was observed three days after the occlusion. This indicates that a pressure as low as 15 to 32 mm. Hg for a period of two hours is adequate to protect the kidneys against renal damage.

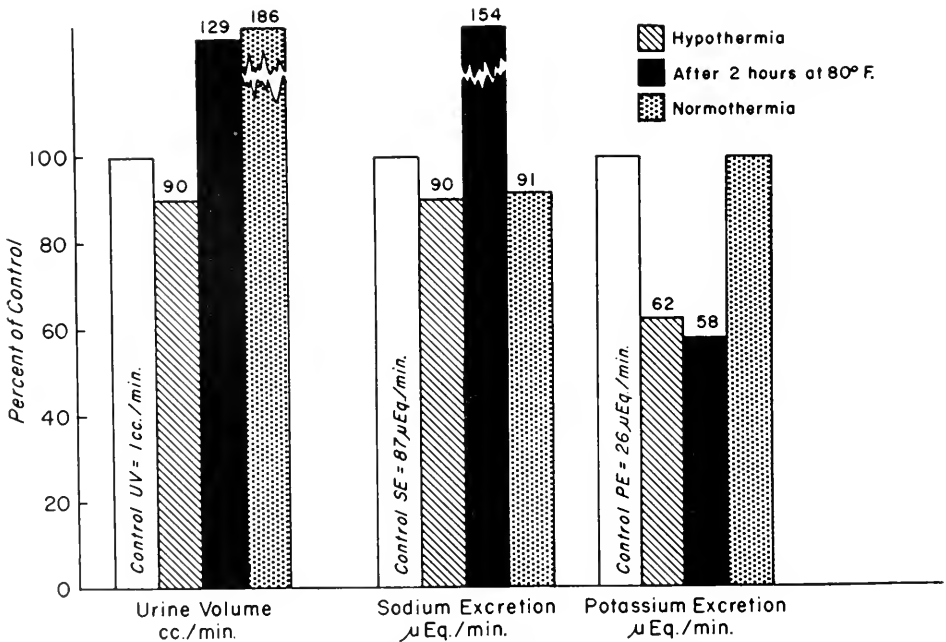


FIG. 5.—The effect of prolonged hypothermia on urine and electrolyte excretion as compared to the immediate response to temperature reduction.

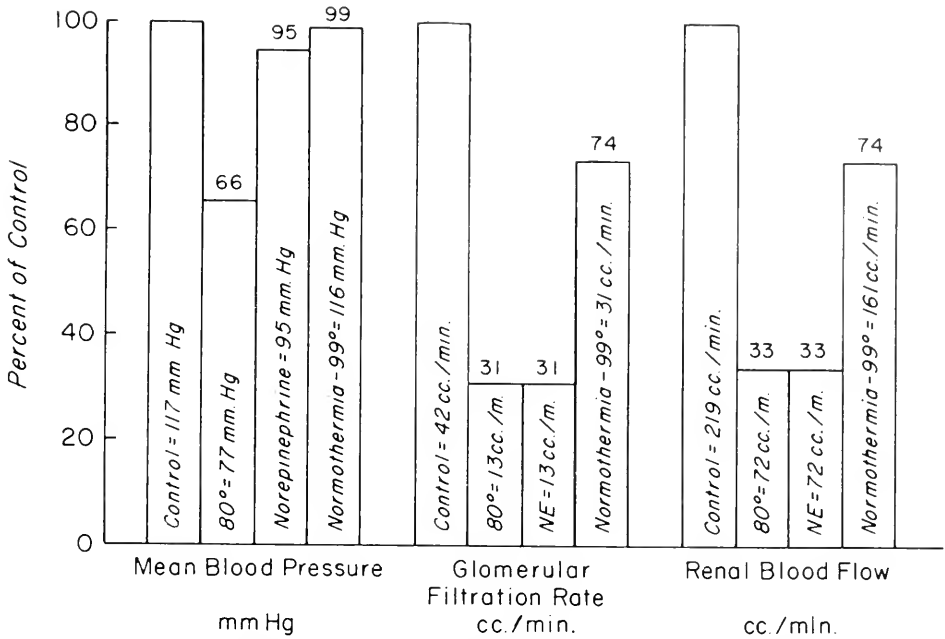


FIG. 6.—The effect of increasing the blood pressure to normotensive levels with norepinephrine during hypothermia. This did not improve glomerular filtration rate nor did it increase renal blood flow.

The important effects of ischemia on renal hemodynamics produced by renal artery occlusion (subgroups 2B and 2C) are summarized in figures 7A and 7B. When only the renal artery was occluded and the aorta was left intact (group 2B), observations three days later indicated that glomerular filtration rate and renal blood flow were reduced to about $\frac{2}{3}$ of the control values (68 and 60 per cent respectively). There was no effect on plasma-sodium and potassium. The unoccluded kidney (control kidney) showed no alterations in renal function for the group.

When both the aorta and renal artery were occluded (group 2C), the left kidney (occluded kidney) was damaged severely but the right kidney (unoccluded kidney) was not damaged. Three days after the occlusion, the glomerular filtration rate in the left kidney (occluded) was reduced to 8 per cent of the control levels and renal blood flow to 7 per cent. At the same time, glomerular filtration rate in the right kidney was 100 per cent of the control and the renal blood flow was 99 per cent of the control value. Under these circumstances the right kidney served as a control. A combination of renal artery occlusion and occlusion of the aorta produced the greatest renal damage in the occluded kidney (left) of any of the methods employed. This is probably due to a reduction in blood flow to the kidney through the renal artery as well as collateral flow through the renal cortex via the lumbar vessels and perhaps through the adrenal gland. These observations also indicate that very low pressures (below 32 mm. Hg) have a protective effect on the kidney since the right kidney was not damaged as a result of the occlusion of the aorta.

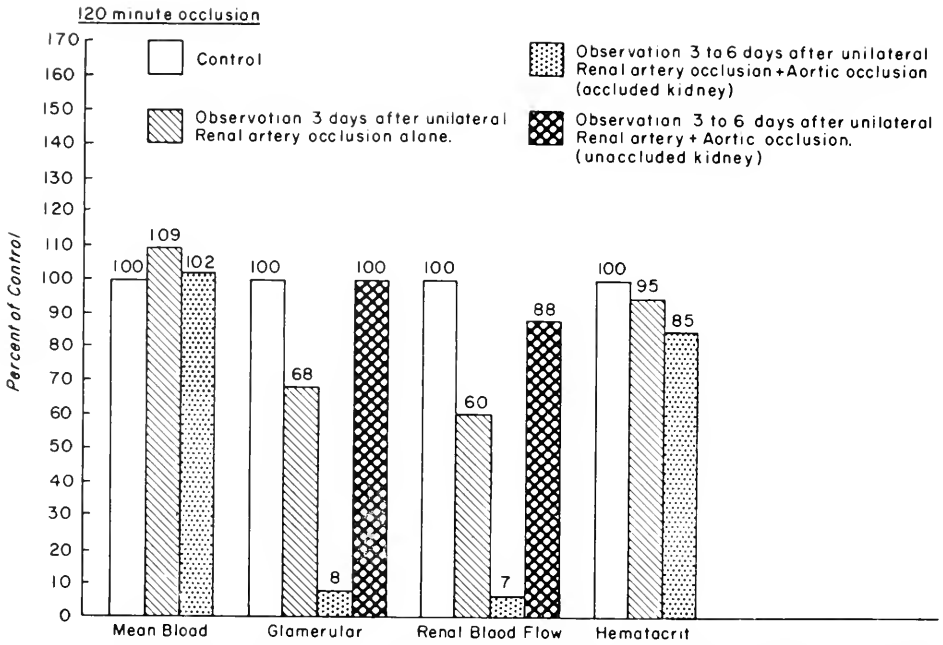


FIG. 7A.—The effect of unilateral renal artery (left) occlusion alone (Group 2B) for two hours as compared to the effect when the renal artery was occluded as well as the aorta (Group 2C).

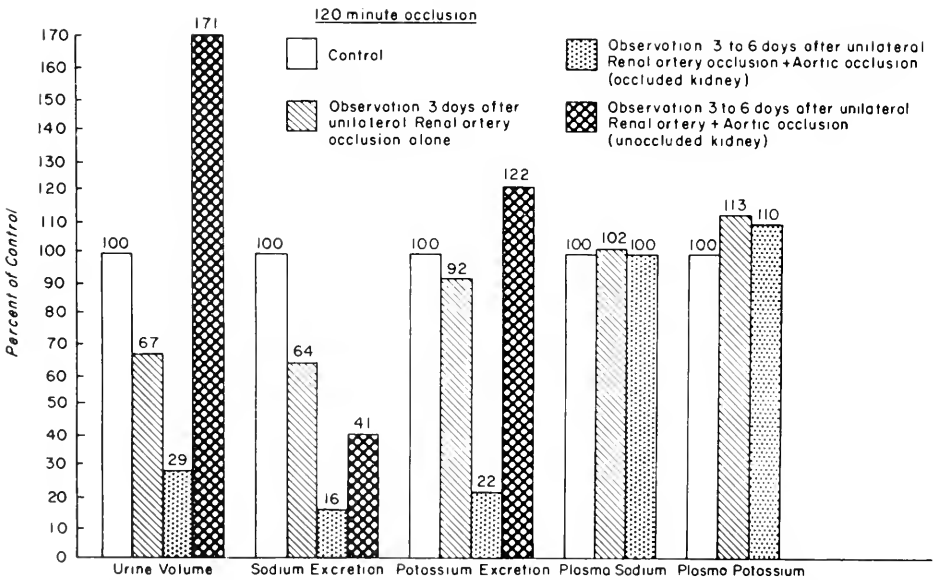


FIG. 7B.—The effect of renal ischemia on water and electrolyte excretion. The ischemia was produced by the two methods indicated in figure 7A.

The responses of sodium potassium and water excretion of subgroup 2C were similar to the renal hemodynamic effects. In the kidney in which the renal artery was occluded as well as the aorta, urine output and sodium excretion were markedly depressed after three days. At the same time, urine volume actually increased in the unoccluded kidney, i.e., the kidney which was ischemic only to the extent of having the aorta occluded. There was no effect on plasma sodium and potassium.

The effect of renal ischemia during hypothermia. Since concurrent occlusion of the left renal artery and the aorta produced the most severe renal damage under normothermic conditions (group 2C), this method of producing ischemia was next used for studying the protective effect of hypothermia against renal damage due to renal ischemia. It appears that even with hypothermia for two hours, some depression in renal function occurs, but the degree of renal damage is not as severe as that observed following ischemia produced by the same method under normothermic conditions. Table I points up the sharp contrast in the observations made under normothermic conditions when glomerular filtration rate was depressed to 8 per cent of the control value and renal blood flow to 7 per cent. These observations indicate that hypothermia would be advantageous when complete occlusion of the circulation to the kidney becomes a necessity during surgical procedures. The protective effect of hypothermia is also seen in figures 8, 9, 10, and 11. These are the gross changes produced under normothermic as compared to hypothermic conditions for an equal period of time. The right kidney was subjected to ischemia due to aortic occlusion above the renal arteries whereas the left kidney was subjected to ischemia produced by occlusion of the renal artery as well as the aorta. Under normothermic conditions the left kidney showed severe damage with hemorrhage into both the cortex and medulla, by comparison to the right one. However, under hypothermic conditions there was very little difference observed between the left and right kidneys.

SUMMARY—LABORATORY DATA

Observations have been made in the laboratory on the effect of hypothermia at different levels of temperature reduction as well as the effect of prolonged hypothermia. As the temperature is reduced, there is a progressive reduction in mean blood pressure, glomerular filtration rate and renal blood flow. The reduction in glomerular filtration rate is not associated with a concurrent reduction in water and

TABLE I

COMPARISON OF RENAL DAMAGE DUE TO ISCHEMIA (AORTA PLUS RENAL ARTERY OCCLUSION) WITH AND WITHOUT HYPOTHERMIA—EXPRESSED IN PER CENT OF CONTROL

	Normothermia		Hypothermia	
	Right (unoccluded)	Left (occluded) ^a	Right (unoccluded)	Left (occluded) ^a
Mean blood pressure.....	102	102	95	95
Glomerular filtration rate.....	100	8 ^b	96	69
Renal blood flow.....	88	7 ^b	115	80
Hematocrit	85	85	106	106

^a Renal artery occluded in addition to occlusion of aorta above the renal artery for two hours.

^b Statistically significant $p < 0.05$.

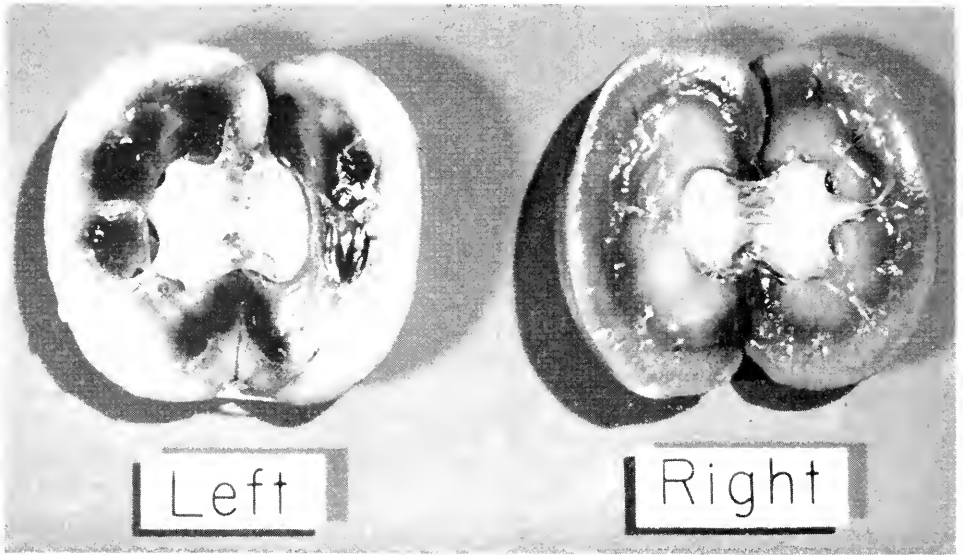


FIG. 8.—Gross changes observed in the kidneys removed from a dog three days after the aorta and the renal artery to the left kidney were occluded for a period of two hours under normothermic conditions.

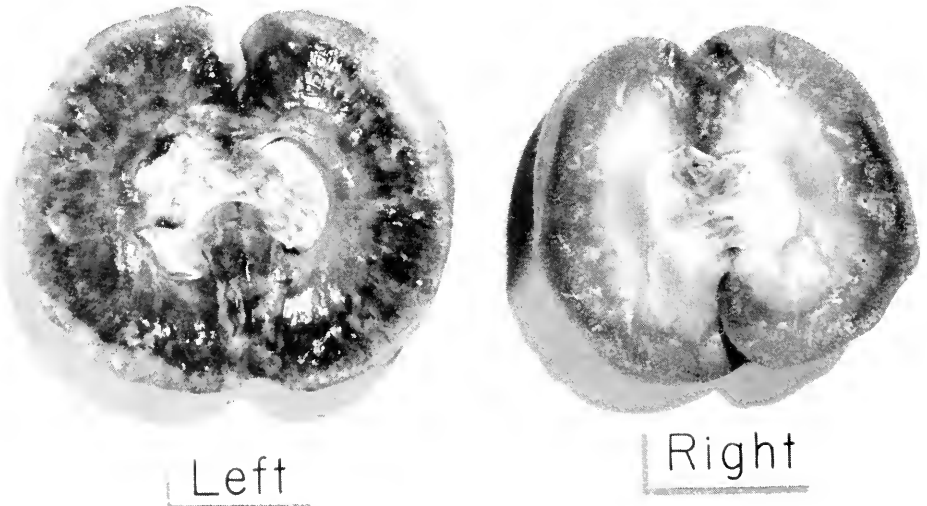


FIG. 9.—Another animal treated as in figure 8.

sodium excretion but there is a depression in potassium excretion. These responses are just as marked immediately after reduction in temperature as they are after hypothermia for two hours or more. Raising the blood pressure to control levels during hypothermia does not improve glomerular filtration rate or renal blood flow, indicating that the depression in renal hemodynamics is a response to hypothermia rather than being secondary to the reduction in blood pressure.

Observations have been made on the effect of renal ischemia produced by three

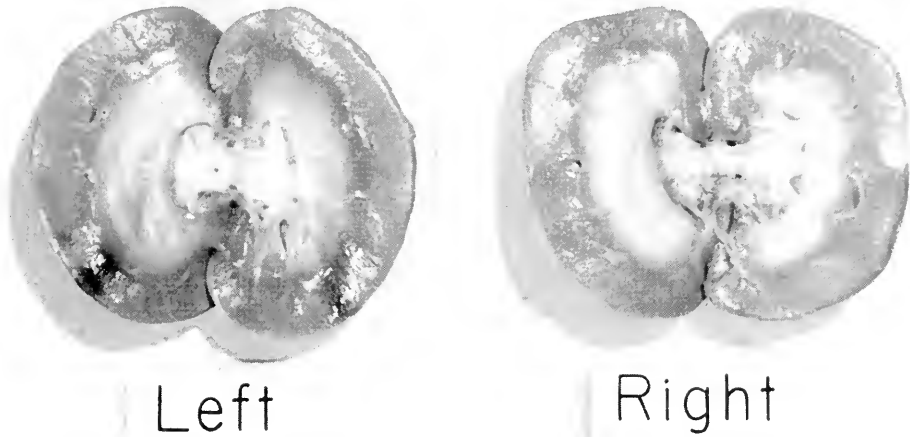


FIG. 10.—Gross effects of occlusion of the aorta above the renal arteries as well as the left renal artery for two hours under hypothermic conditions. When compared to figures 8 and 9, there is relatively little damage to the left kidney of these animals under hypothermia as compared to the normothermic animals.

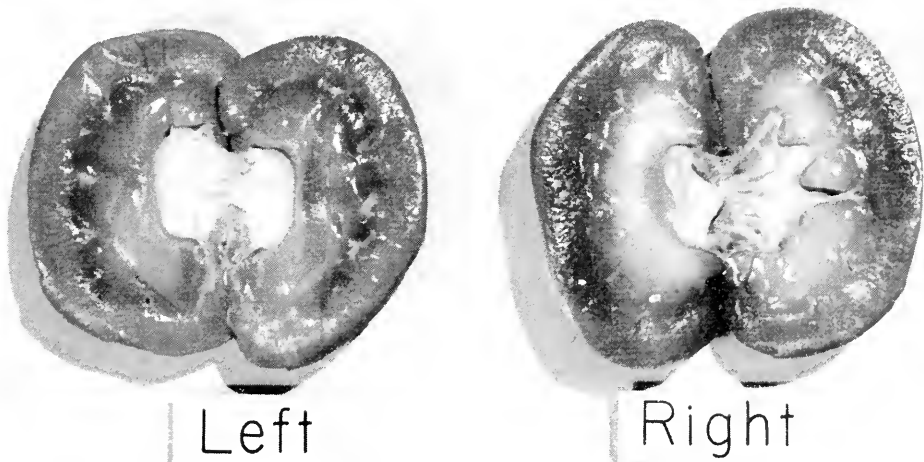


FIG. 11.—Another animal treated as in figure 10.

different methods. In one group, the response to occlusion of the aorta was observed. In another group of animals, the effect of occlusion of the renal artery to one kidney was studied. In the third group the effect of occlusion of the renal artery plus the aorta was observed. Collateral circulation and retrograde flow through the inferior aorta was adequate to protect the kidney from damage following occlusion of the aorta immediately above the renal arteries. After two hours of occlusion of the renal artery alone, the over-all depression in renal function of that kidney showed about a $\frac{1}{3}$ reduction in glomerular filtration rate and renal blood flow. However, if the renal artery and the aorta were occluded together,

renal damage was severe. Glomerular filtration rate and renal blood flow in the occluded kidney were then reduced to about 10 per cent of the contralateral kidney. In fact, there was no renal damage on the contralateral side, again indicating that a low pressure in the aorta for two hours was adequate to protect the kidney from damage due to inadequate circulation.

When hypothermia was employed during renal ischemia, it appeared that moderate protection was given to the kidney. Observations made three days after the renal artery and aortic occlusion indicated that the renal function was depressed overall only to about $\frac{2}{3}$ of the control level as compared to 10 per cent of the control under normothermic conditions. These observations indicate that under states of severe ischemia, hypothermia gives some protection against severe renal damage.

OBSERVATIONS IN MAN

Methods. Observations on renal function were made in four patients in whom hypothermia was employed for resectional therapy of aortic aneurysms. The temperature was reduced to 88° to 91° F. The primary purpose of the hypothermia was to prevent ischemic spinal cord damage during cross clamping of the descending thoracic aorta. Observations on renal function were made before and after induction of anesthesia. Total body cooling was then induced and observations on renal function were repeated. During the surgical procedure renal function was measured at approximately the mid-period of aortic occlusion and immediately after release of the occluding clamps. Renal function was again determined after rewarming and, in two cases, one week following operation. Inulin clearance was used to measure glomerular filtration rate (GFR) and low concentrations (2 to 4 mg. per cent) of para-aminohippurate to measure renal plasma flow (RPF) employing methods and techniques previously described. (2). Values listed in the tables and figures are averages of two or three 10-minute collecting periods.

RESULTS AND DISCUSSION

Due to the inherent difficulty in making these observations on patients during hypothermia and surgery, a relatively small number of patients were studied. However, as detailed information was obtained, the data are presented as case reports demonstrating pertinent points.

CASE 1.—A 66-year-old Negro male had a thoraco-abdominal aneurysm involving the celiac axis, the superior mesenteric and both renal arteries. The (rectal) temperature was reduced to 90° F. employing refrigeration blankets. The aneurysm was resected and replaced with a lyophilized homograft extending from the lower thoracic aorta to the abdominal aorta distal to the renal arteries. The period of aortic and bilateral renal artery occlusion was one hour and forty minutes.

Figure 12 graphically demonstrates the effects of these procedures on renal function. The control values showed a slightly depressed renal blood flow and glomerular filtration rate. Pentothal anesthesia did not produce any striking changes. As in the experiments with dogs, there was some depression in both renal blood flow and glomerular filtration rate associated with an increase in urine volume.

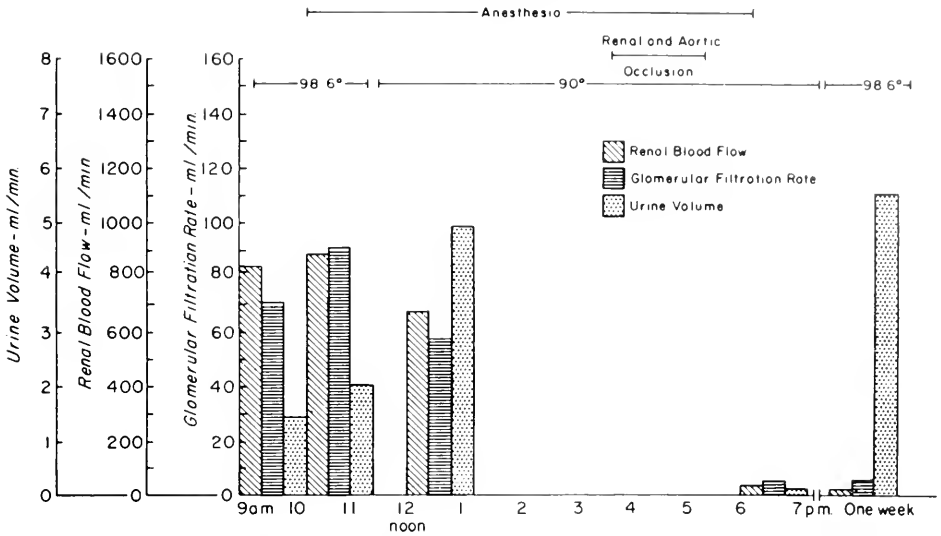


FIG. 12.—The effect of anesthesia, hypothermia and prolonged ischemia of the kidney under hypothermia during surgical homographic replacement of the aorta.

During aortic and renal artery occlusion there was no measurable renal function. After rewarming, an extreme depression in both renal function and water excretion was observed. One week following operation, extreme depression in both renal blood flow and glomerular filtration rate persisted due to the severe renal damage which occurred during occlusion of the renal arteries and aorta. Urine volume at this time was increased, which probably reflects tubular damage with failure of concentration.

CASE 2.—A 54-year-old white male had a dissecting aneurysm involving the descending thoracic aorta. He was cooled to a temperature of 89° F. with refrigeration blankets. The point of dissection began just distal to the left subclavian artery and extended below the diaphragm. A portion of the descending thoracic aorta was resected and replaced with a lyophilized homograft. The period of aortic occlusion was 53 minutes.

As presented in table II the control values for this patient show moderate hyper-

TABLE II

RENAL HEMODYNAMIC RESPONSE TO HYPOTHERMIA AND AORTIC OCCLUSION

	Blood pressure (mm. Hg)	Renal blood flow (ml/min.)	Glomerular filtration rate (ml/min.)	Urine volume (ml/min.)
Control observations (99° F.)	160/100	1126	109	.4
Anesthesia (pentothal)	150/100	1175	139	1.6
Hypothermia (94° F.)	130/90	1026	95	5.6
Hypothermia (89° F.)	100/70	985	81	2.4
Aortic occlusion (89° F.)	100/80	27	1	0.1
After occlusion (89° F.)	130/80	545	25	1.1
Rewarmed (98° F.)	200/90	866	82	2.7
After 7 days (98° F.)	172/102	771	91	2.1

tension and renal function within normal range. There was a slight increase in glomerular filtration rate during pentothal anesthesia. At 94° F. there was a slight depression in blood pressure, renal blood flow and glomerular filtration rate. There was an increase in urine volume probably due to decreased tubular reabsorption of glomerular filtrate resulting from the hypothermia. A further moderate depression in blood pressure and renal function occurred when the rectal temperature was reduced to 89° F. During aortic occlusion, renal function was nil. Immediately after release of the clamps, renal function partially returned but remained much below the preclamping, hypothermic level. After rewarming, renal function was largely restored though still somewhat below control values. A week later, a slight depression was still evident.

CASE 3.—A 56-year-old white male had a fusiform aneurysm of the descending thoracic aorta. The aneurysm was resected and replaced with a lyophilized homograft. Hypothermia to a level of 88° F. was carried out by ice water immersion under pentothal anesthesia. The period of aortic occlusion was 40 minutes.

As illustrated in figure 13, there was no significant change in renal function during anesthesia. During hypothermia, renal blood flow and glomerular filtration rate were reduced almost 50 per cent. The usual relative increase in urine volume was not observed until the patient was partially rewarmed. No determinable function was present during aortic occlusion. Immediately after rewarming, renal function approached control values.

CASE 4.—A 36-year-old Negro female had a fusiform aneurysm of the descending thoracic aorta. Hypothermia was induced with refrigeration blankets and the aneurysm was resected and replaced with a lyophilized homograft. The period of aortic occlusion was 35 minutes with a rectal temperature of 91° F. (table III).

Again, in this patient, the characteristic moderate reduction in renal function

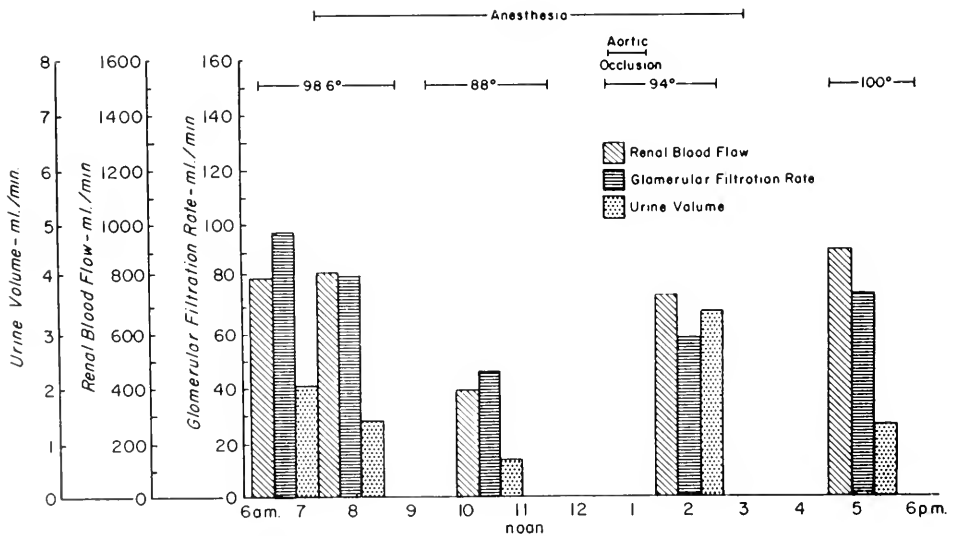


FIG. 13.—The effect of anesthesia, hypothermia and aortic occlusion under hypothermia on renal function in a 56-year-old white male who had a homograft replacement of the aorta.

TABLE III

	Blood pressure (mm. Hg)	Renal blood flow (ml/min.)	Glomerular filtration rate (ml/min.)	Urine volume (ml/min.)
Control observations (98° F.).....	130/80	1253	90	.9
Hypothermia (91° F.)	100/80	782	72	.8
Aortic occlusion (91° F.).....	130/80	0	0	0
After occlusion (91° F.).....	124/80	447	37	1.4
Rewarmed (100° F.)	128/80	920	104	.8

was seen with hypothermia. The urine volume, however, was not significantly reduced and was increased after occlusion while still hypothermic. Again, return in function was incomplete immediately after release of the clamps compared to pre-occlusion hypothermic values. However, after rewarming, renal function approximated the control observations.

SUMMARY

It was found that the renal functional response of these four patients to hypothermia was similar to that of the dog. Glomerular filtration and renal blood flow were moderately depressed. These returned to or towards control levels with normothermia. Hypothermia did not prevent renal damage due to ischemia if the period of renal artery and aortic occlusion was prolonged. In the one case of combined aortic and renal artery occlusion for an extended period of time, severe irreversible renal damage occurred in spite of hypothermia.

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EFFECT OF HYPOTHERMIA ON THE KIDNEY

R. K. ANDJUS

I would like to summarize some of my investigations concerning the renal function, especially the electrolyte excretion and the effect of hypothermia on the re-absorption capacity of the renal tubules.

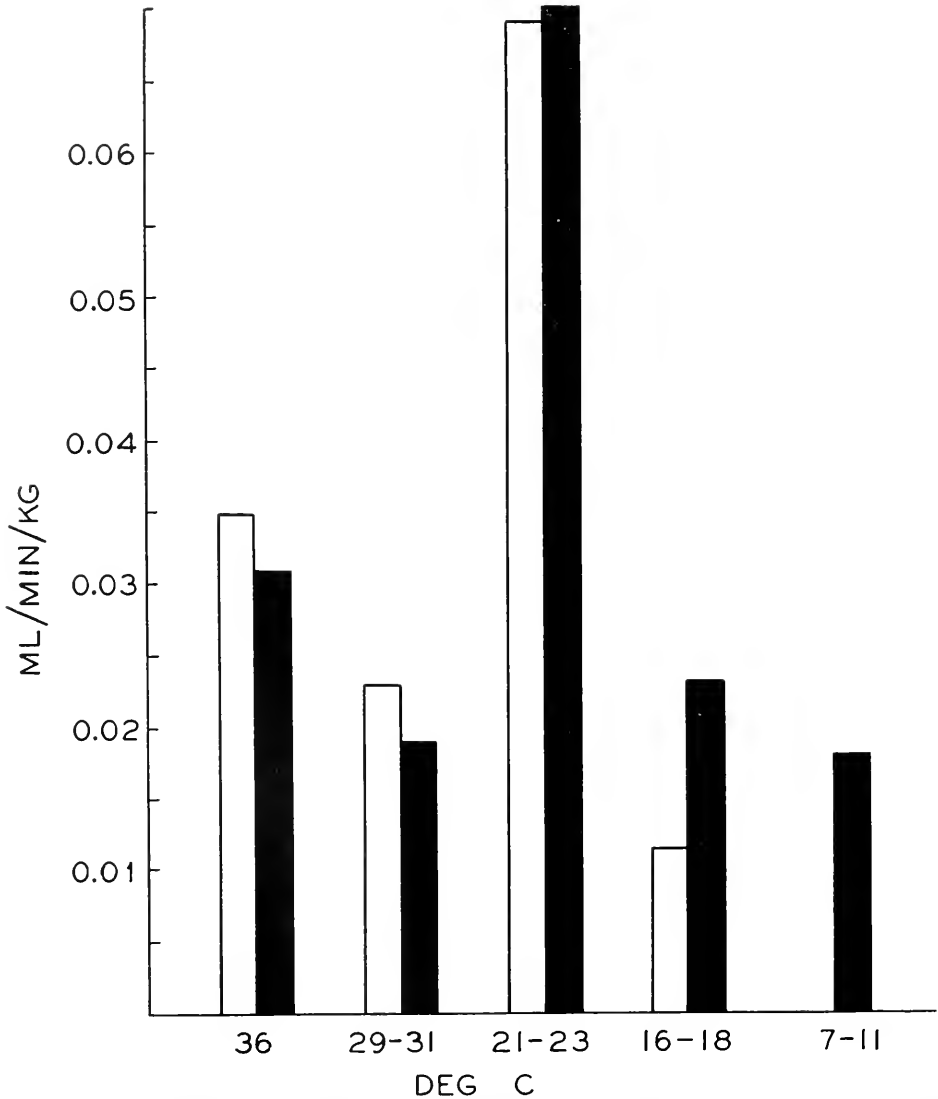


FIG. 1.—Spontaneous urine flow in rats (white bars) and ground squirrels (black bars) during 60 minutes at various body temperatures. Each bar represents the average value from 5 experiments. Results obtained at different levels of hypothermia were obtained with different groups of animals.

Sodium excretion was recorded with injected tracer sodium (Na^{24}) by cannulating the bladder and making continuous kymograph tracings of the radioactivity of the outflowing urine. The lowest temperature reached in these experiments was 0°C . Similar experiments were performed on a hibernating mammal, the ground squirrel (*Citellus citellus*).

The excretion of urine continues in the rat until body temperatures below 18°C . are reached. Moreover, at temperatures of about $20\text{--}23^{\circ}\text{C}$., an increased urinary output has been often recorded (Andjus and Morel, 1952). Similar results were obtained in ground squirrels (artificially cooled in the same manner), except that the temperature limit for diuresis may reach as low as 7°C . of body temperature (fig. 1). The maximal intensity of osmotic diuresis as influenced by the level of hypothermia in rats and ground squirrels is shown in figure 2.

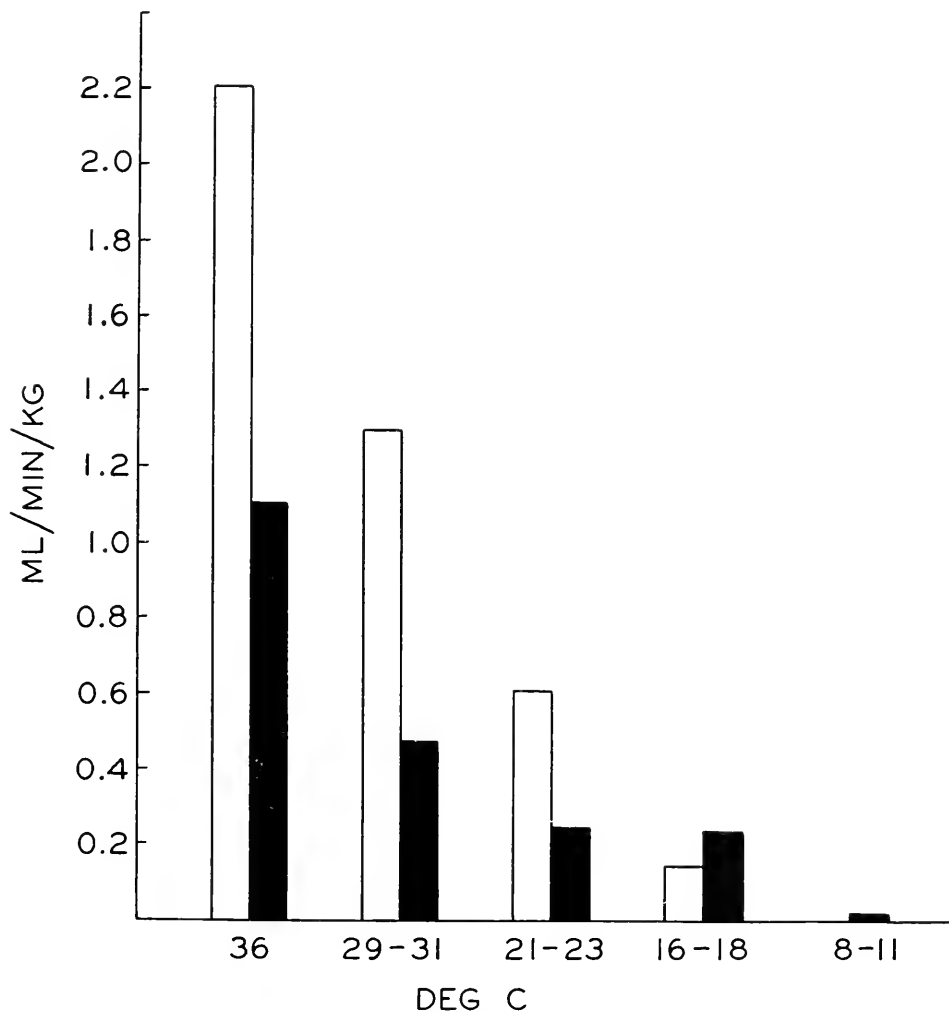


FIG. 2.—Maximal intensity of osmotic diuresis after an intravenous injection of 2 ml. of a 50 per cent glucose solution into rats (white bars) and ground squirrels (black bars).

During cooling (immersion in water, light nembutal anesthesia), the concentration of Na^{24} does not change appreciably in either the blood or in the urine. On rewarming, however, usually when body temperatures over 30°C . are reached, a marked fall of the urinary concentration of sodium occurs (fig. 3). The disappearance of sodium from the urine toward the end of rewarming seems to be independent of the antidiuretic hormone and some facts indicate that it may be related to the hyperglycemia and glucosuria encountered in hypothermia (Andjus and Morel, 1952).

In order to study the effect of hypothermia on the reabsorption capacity of the renal tubules, two different ways of eliciting maximal reabsorption activity were used. Maximal tubular reabsorption of sodium and consequently its disappearance from the urine can be induced in rats either (1) by injecting hypertonic glucose intravenously, or (2) by eliminating the antidiuretic hormone from the circulation (temporarily, as in water diuresis, or permanently, as in operative diabetes insipidus).

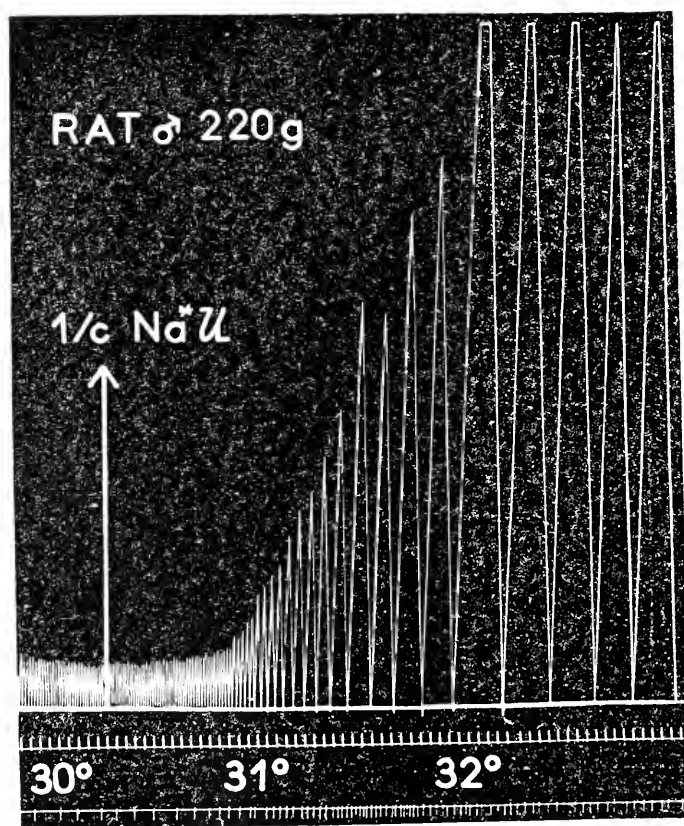


FIG. 3.—Spontaneous fall of sodium concentration in the urine toward the end of rewarming of a rat cooled to 15°C . (Andjus and Morel, 1952). From above downward: (1) Radioactivity of the urine. The peaks are inversely proportional to the actual concentration of radio-sodium in the outflowing urine (cNa U). (2) Time (minutes). (3) The rectal temperature. (4) Drops of urine.

The first type of experiment consisted of recording the urinary sodium concentration after an intravenous injection of 50 per cent glucose solution to rats kept at different levels of hypothermia. Osmotic diuresis resulted, provided the body temperature did not fall below 18–20° C. This was characteristically followed by a marked sodium retention at body temperatures above 23° C. (fig. 4). At lower temperatures, the osmotic diuresis was not followed by the disappearance of sodium from urine, indicating possible failure of the sodium reabsorption mechanism (fig. 5). Similar results were obtained by eliciting glucosuria and osmotic diuresis with phloridzin.

The second type of experiment consisted of studying sodium excretion in hypothermic rats deprived of the antidiuretic hormone. It was first found that it is impossible by injecting water to obtain a typical water diuresis accompanied by the disappearance of sodium from urine in hypothermic animals below 30° C. of body temperature. Moreover, when a typical diuresis following water injection is obtained at normal body temperatures, it ceases promptly when such an animal is subjected to cooling. The urinary concentration of sodium rises to that of plasma levels by the time the body temperature falls below 25° C. Failure to induce sodium retention by a water load in hypothermic animals and the interruption of this retention by cooling could have been the consequence of a release of ADH elicited by the cooling procedure, or of some other effect of low temperature on the hormonal mechanism regulating the function of renal tubules. In order to see, however, whether hypothermia has a direct effect on the reabsorption capacity of the tubules, further experiments were performed on rats with permanent diabetes insipidus provoked by hypothalamic lesions. These animals, deprived permanently

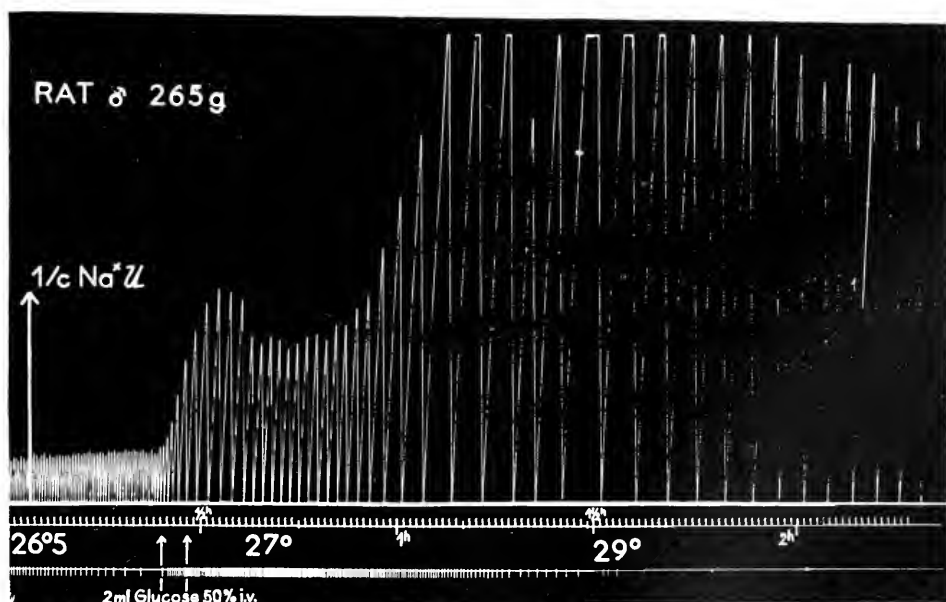


FIG. 4.—Osmotic diuresis in a rat maintained at 26–29° C. of body temperature after an intravenous injection of 2 ml. of 50 per cent glucose solution. Marking as before (Andjus and Morel, 1952).

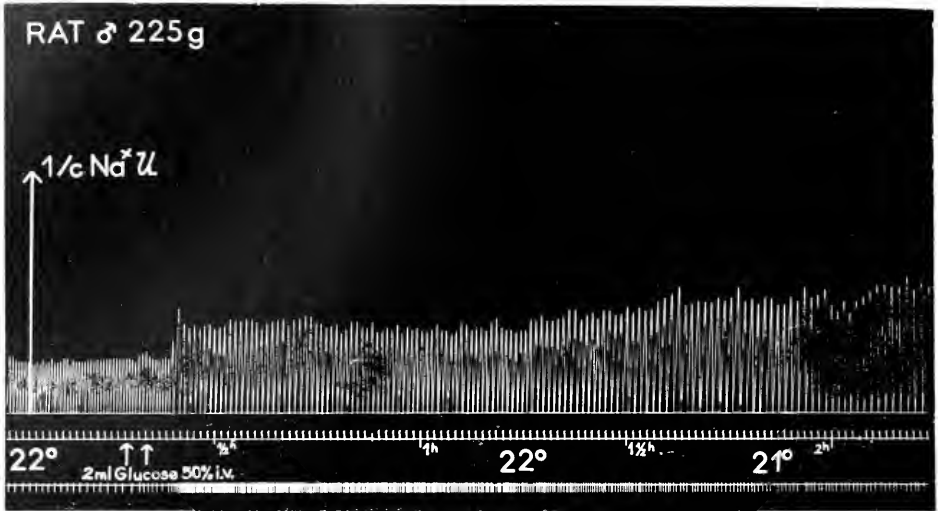


FIG. 5.—Osmotic diuresis in a rat maintained at 21–22° C. of body temperature after the same hypertonic load as in the experiment illustrated by figure 4.

of ADH, and showing a continuous maximal sodium retention at normal body temperature, failed to retain sodium when cooled to below 23° C. The urinary concentration of Na^{24} rose to plasma levels (fig. 6).

These results, which are in accordance with the data obtained by Bickford and Winton (1937) on the isolated kidney, speak in favor of a direct inhibitory effect of hypothermia on the reabsorption activity of the renal tubules. In a range of temperatures between 18 and 23° C., sodium reabsorption is completely inhibited, while glomerular filtration and urinary flow are still present. Such a reversible dissociation of the kidney function suggests the use of the hypothermic animal as a special experimental preparation for the renal physiologist.

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DISCUSSION

Capt. Philip A. Riley, MC: At the Walter Reed Army Institute of Research we have studied urine production and composition during hypothermia to 20° C. in dogs, and noted several interesting changes.

The minute volume of urine excreted increased progressively as the animals were cooled, despite a marked reduction in filtration rate. This increase in urine flow was unaltered by antidiuretic hormone.

The urine composition was also affected by the decrease in temperature. The urine became progressively more alkaline as cooling progressed until it approached

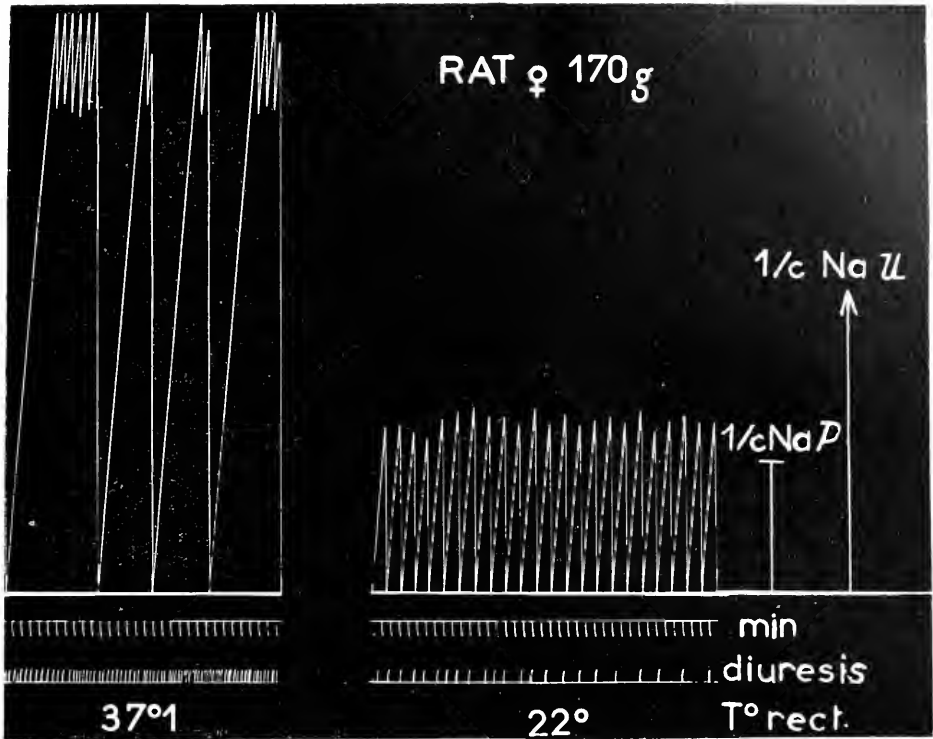


FIG. 6.—Effect of hypothermia on spontaneous urine flow in a rat with permanent diabetes insipidus (hypothalamic lesion). “cNa P” = plasma concentration of radio-sodium; “cNa U” = urinary concentration of radio-sodium. (The zig-zag line on the top of the radioactivity tracing at 37° C. is the consequence of a mechanical arrangement preventing the excursion of the writing pen over the upper border of the smoked drum.)

the pH of whole blood. During warming the urine again became more acid. There was very little titratable acid at any time. It should be pointed out that our animals were mechanically ventilated throughout the experiment with room air at what was calculated to be a normal minute volume. Ammonia production was sharply decreased during cooling. Initial urines contained an average of 48.6 mg. per cent and at the depth of cooling 2.7 mg. per cent was present. This function returned as the dogs were warmed. The concentration of Na and Cl in the urine increased during cooling to values closely approximating those of the serum, and returned to normal during rewarming. Potassium concentration in the urine fell rapidly with cooling to a value only three times that of the serum. The concentration of creatinine in the urine fell from 116.9 mg. per cent initially to 7.8 mg. per cent with cooling.

Creatinine clearance fell markedly, but Na and Cl clearance rose as cooling progressed. These were reversed with rewarming. Potassium clearance was unchanged.

Filtration rates were based on endogenous creatinine clearance. The percent excretion of each filtered load was affected; H₂O, Na and Cl paralleled each other closely from an initial value of 0.5 per cent at 37° C. to 12 per cent at 22° C. and

returned to normal with warming. In contrast, 9 per cent of filtered potassium was excreted at 37° C., while 36 per cent was excreted at 22° C. This returned to normal with rewarming.

The urine composition thus assumed the composition of plasma ultrafiltrate where U/P ratios approached unity as cooling progressed. The clearance of substances normally reabsorbed by the kidney tubules, such as H₂O, Na and Cl were increased, while clearances of nonreabsorbable solutes such as endogenous or exogenous creatinine were reduced. The clearances of substances believed to be secreted by the tubular cells were also reduced.

We therefore believe that distal tubular function is greatly reduced during cooling and nearly eliminated at 22° C., while proximal tubular activity is little affected by hypothermia.

HYPOTHERMIA AND TEMPORARY OCCLUSION OF THE HEPATIC CIRCULATION

NORMAN E. SHUMWAY* AND F. JOHN LEWIS

A need has long existed for increasing the scope of cancer surgery to include ablation of the right lobe of the liver. Lortat-Jacob in 1952 reported the first successful right hepatic lobectomy.¹ Since then, a growing literature has outlined a variety of techniques for excising the right lobe of the liver.^{2, 3, 4, 5} Despite the efforts of Elias to ascribe a segmental unit to the surgical anatomy of the human liver,⁶ extirpation of the right lobe necessarily involves cutting across a large vascular area. It is clear that the hazard of extreme blood loss from this maneuver could be circumvented by occluding temporarily both the afferent and efferent vasculature of the liver.

Hypothermia has been demonstrated to reduce splanchnic blood flow and liver function without significant deleterious effects related to cold;⁷ hence, the use of hypothermia to gain sufficient time for massive hepatic resections on a bloodless liver seemed a promising avenue for investigation.

That the experimental animal at normal temperature can tolerate only brief periods of occlusion of the structures in the porta hepatis has been recognized for years.⁸ The relative importance of the hepatic artery and portal vein and the specific role of each are problems still under scrutiny although considerable information is now at hand.

Wolbach in 1909 described the presence of anaerobic, spore-bearing bacteria in the normal canine liver.⁹ This work permitted Markowitz ultimately to assign to the hepatic artery in the dog the function of maintaining oxygen tension in the liver high enough to prevent proliferation of bacteria.¹⁰ Successful transfer of this bacteriostatic function to penicillin after ligation of the hepatic artery was the crucial laboratory evidence.¹¹

Doubtless the portal vein can contribute oxygen to hepatocellular oxygen tension in the dog because some animals will survive hepatic artery occlusion without penicillin.¹² In these dogs arterial blood reaches the liver via hepatic branches of the phrenic arteries, but subsequent occlusion of the portal vein results in death and indicates that the contribution of oxygen from portal venous blood may be critical under such circumstances. Nonetheless, protection of the dog from death due to liver failure depends primarily on the integrity of the hepatic artery while occlusion of the portal vein, though harmless to the liver, is disastrous because of intestinal infarction and exsanguination of the dog into his abdominal viscera.

In the absence of any feasible method for bypassing the liver it is necessary to occlude not only the hepatic artery and portal vein but also the vena cava both above and below the liver in order to secure a bloodless operative field and prevent air embolism to the heart. Occlusion of the inferior vena cava above the liver is not tolerated for more than a few minutes unless the aorta is clamped at the same or preferably at a higher level. Obstruction of the thoracic aorta introduces, then,

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the advisability of employing hypothermia to lower the blood pressure and cardiac output, slow the heart rate, and reduce the oxygen requirement particularly of nerve tissue distal of the point of aortic occlusion. What was at the outset a problem of interruption of the hepatic circulation is for practical purposes a study of the ischemic response of the animal to occlusion of the descending thoracic aorta.

Method and Results. Three groups of experiments were performed on adult mongrel dogs of either sex, anesthetized with sodium pentobarbital, and subjected to the following technique of hepatic circulatory obstruction. Through a transverse thoracoabdominal incision the chest and peritoneal cavities were entered between the 10th and 11th ribs on the right. The diaphragm was incised to the level of the vena cava. Twill tapes were placed around the vena cava above the diaphragm and below the liver. The hepatic artery and portal vein were occluded with a noncrushing clamp after the aorta had been clamped at the level of the 10th intercostal space. Next, the caval tourniquet below the liver was secured, and finally the supradiaphragmatic cava was occluded. Theoretically this technique totally separated the liver from its circulation. All animals received penicillin postoperatively.

Dogs in Groups II and III underwent partial right hepatic lobectomy. Because the dog liver conforms more closely to a lobular pattern than does the human, a large divot was purposely sliced from the right lobe to create a raw surface of generous proportions which included numerous holes in the vena cava. No effort was made to ligate individually at the hilus those members of the portal triad afferent to the right lobe. The amount of bleeding varied, but the presence of considerable quantities of bright red blood encountered in all resections led to the inescapable conclusion that the collateral circulation of the dog liver is extensive and endures even after supradiaphragmatic occlusion of the aorta. In this respect the dog liver resists attempts to render it totally ischemic almost as vigorously as does the dog brain. Cardiac stasis is probably the only method of insuring absolute cerebral circulatory arrest! After suture ligation of all vessels and bile ducts on the cut surface of the liver and repair of the vena cava, the occlusion was released in reverse order to that of application. Bleeding sites obscured by the occlusion were then evident and dealt with accordingly. Finally the raw area was covered with Gelfoam and the incision closed without drainage.

Group I. Four dogs were submitted to hepatic circulatory occlusion for one hour at normal temperature. No liver resection was performed. Table I lists the results. Shortly before demise two animals developed massive melena. The two survivors were free of neurological deficit.

Group II. Six dogs underwent hepatic resection of the right lobe during 40 minutes of occlusion at normal temperature. Results are reviewed in table II. Three of the four survivors had transient hind limb weakness. The two deaths

TABLE I

OCCLUSION OF HEPATIC CIRCULATION AT NORMAL TEMPERATURE

No. of dogs	Duration of occlusion	Remarks
4	60 minutes	2 died, death preceded by melena 2 survived

TABLE II

OCCLUSION AND HEPATIC RESECTION AT NORMAL TEMPERATURE

No. of dogs	Duration of occlusion	Remarks
6	40 minutes	4 survivors, 3 with neuro deficit Neither death due to hemorrhage from operative site

occurred in the immediate postoperative period; neither was due to hemorrhage from the raw surface of the liver.

Group III. Twelve dogs were subjected to hepatic resection and 20 to 40 minutes of occlusion under hypothermia. Results are seen in table III. Hypothermia of 22° to 28° C. was induced by ice water immersion, and hot water at 45° C. was used for rewarming. Artificial respiration was maintained throughout both the cooling and rewarming procedures. Ventricular fibrillation did not occur, and there were no deaths either early or late.

Discussion of experimental results. Finding arterial blood in the process of performing hepatic resections could be predicted from the first group of experiments. The liver became engorged and cyanotic toward the end of the hour. The intestines also participated in the sequestration of blood from collaterals since dilatation of the intestinal veins was considerable. In one experiment at the end of 30 minutes portal pressure had quadrupled. To a lesser, but still notable, extent the inferior vena cava below the liver increased in size during the period of occlusion.

In all experiments heart action became progressively less vigorous, and the heart rate increased. This cardiac response could well have been a result of blood lost through collateral channels into a field from which it could not return due to obstruction of the inferior vena cava.

The fact that some dogs at normal temperature manifested hind limb weakness and not others is probably attributable to the variation in collateral circulation to that portion of the spinal cord below the site of aortic occlusion. None of the dogs in the hypothermia group had hind limb signs.

Whether or not hypothermia is necessary to protect the liver during a period of arterial occlusion is an unresolved problem. In experiments designed to establish the mechanism of death from thoracic aortic occlusion, Edwards concluded that hepatic ischemia was not the lethal factor since maintenance of normal arterial supply to the liver did not affect a 60 per cent mortality associated with 90 minutes of aortic occlusion in the dog.¹³ The fact remains that obstruction of the portal vein for 30 minutes or longer is lethal for the dog because of hemorrhagic intestinal

TABLE III

OCCLUSION AND HEPATIC RESECTION UNDER HYPOTHERMIA

No. of dogs	Duration of occlusion	Remarks				
12	<table border="0"> <tr> <td rowspan="3" style="vertical-align: middle;">{</td> <td>20 minutes-1</td> </tr> <tr> <td>35 minutes-3</td> </tr> <tr> <td>40 minutes-8</td> </tr> </table>	{	20 minutes-1	35 minutes-3	40 minutes-8	12 survivors, no neuro deficit Lowest temp. 22° C., highest 28° C.
{	20 minutes-1					
	35 minutes-3					
	40 minutes-8					

infarction.¹⁴ Occlusion of the inferior vena cava above the liver, necessary for a so-called bloodless field and the prevention of air embolism, must be accompanied either by a shunt or by aortic obstruction to prevent almost immediate shock. Hypothermia permits prolonged thoracic aortic obstruction¹⁵ and is in that sense essential if the contemplated liver resection is to be performed on the isolated organ.

At our institution Raffucci preceded these researches with experiments directed toward establishing the duration of afferent hepatic circulatory occlusion tolerated by dogs. He concluded that 20 minutes was the maximum safe period of portal vein and hepatic artery obstruction under normothermic conditions.^{16, 17} Simultaneous clamping of the superior mesenteric artery was the only measure taken to prevent intestinal engorgement. In the light of our studies it would seem that the deaths attributed to hepatic necrosis by Raffucci were in fact due to the effects of portal obstruction with consequent shock and intestinal infarction. The beneficial or protective effect of hypothermia, however, was clearly shown by Raffucci when only 3 of 11 dogs succumbed after one hour of occlusion of the superior mesenteric artery, celiac axis, portal vein, and hepatic artery.¹⁸ No doubt less blood was isolated from the circulation because of depressed collateral activity in response to hypothermia.

Clinical experience. Table IV is a summary of the results obtained in four patients submitted to total right hepatic lobectomy under hypothermia. The same technique of occlusion was used as in the experimental animals. Cooling, however, was effected by means of refrigerated blankets rather than ice water immersion. The raw surface of the liver after excision of the cancer was notably free of bleeding. There was no evidence of the active collateral arterial supply so noticeable in dogs. There was, however, a much greater postoperative blood loss, probably a function of the increased surface area. One patient bled to death from an hepatic vein which had been ligated but retracted from its ligature. Another patient, M, was re-explored in the immediate postoperative period to secure hemostasis. His course was further complicated by peritonitis, and death ensued 15 days after surgery. Because of difficulties related to bleeding, the fourth patient was rewarmed to 96° F. on the operating table by circulating hot solution through the blankets.

TABLE IV

OCCLUSION AND TOTAL RIGHT HEPATIC LOBECTOMY UNDER HYPOTHERMIA IN HUMANS

Patient	Age	Sex	Diagnosis	Duration of occlusion	Lowest temperature	Remarks
N	9 yrs.	M	Hepatoma	45 minutes	30.5° C.	Expired 8 mos. postoperatively of recurrence
R	6 mos.	F	Hepatoma	33 minutes	31° C.	Expired from hemorrhage in early postoperative period
M	72 yrs.	M	Metastatic carcinoma	40 minutes	30° C.	Expired 15 days postoperatively
K	60 yrs.	F	Metastatic carcinoma	45 minutes	30° C.	Recovered well from procedure but lung metastases found 4 mos. later

The hemodynamic depressant action of hypothermia was thought to have masked multiple small bleeding points encountered in the emergency re-exploration of patient M. Rewarming the patient with the operative field under direct vision discloses any bleeding sites previously undetectable. The operative procedure and postoperative course went very smoothly in the last patient, K.

One final point worth mentioning is that the age factor plays no part in the choice of patients for hypothermia. Two patients were 60 and 72 years old, and their reaction to hypothermia itself was gratifying and without complication.

Summary and Conclusions. Hypothermia is essential to isolate the liver from the circulation primarily because the thoracic aorta must be occluded to maintain circulatory homeostasis.

Resistance of the liver to ischemia is difficult to assay. The problems of "neighborhood" effects incident to the vascular occlusion necessary for rendering the liver ischemic take priority over the hepatic response.

Right hepatic lobectomy can be performed without blocking the liver vasculature, but if hepatic circulatory occlusion is desired, hypothermia must be utilized.

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EFFECT OF HYPOTHERMIA ON METABOLISM AND DRUG DETOXIFICATION IN THE ISOLATED PERFUSED RABBIT LIVER

IRVING GRAY, ROLAND R. RUECKERT AND RICHARD R. RINK

Perfusion of the isolated liver has been used by a number of investigators, particularly in the past five years.¹⁻⁵ Prudden and co-workers⁶ have used this method in the study of the effects of insulin and growth hormone on liver metabolism. Miller *et al.* reported in 1954 that perfusion of the intact-isolated liver with oxygenated blood permits the liver to synthesize plasma proteins, to separate them from its own tissue at need, and to contribute them to the circulating plasma in a manner closely approximating that seen in intact normal animals.⁷ They also reported that the perfused liver repeats its action quantitatively when a second dose of substrate is given, even after having been perfused for four hours.⁷

With the advent of surgery under hypothermic conditions, it was felt that the liver perfusion method for the study of metabolism and detoxification would yield valuable information for this clinical problem. The present study utilized this technique as an *in vitro* method of observing the effect of hypothermia on liver respiration, metabolism and its ability to conjugate morphine and thiopental. In addition to the usual methods for measuring carbon dioxide production and rate of drug detoxification, the apparatus has been so designed as to allow the measurement of oxygen consumption.

Description of apparatus. The liver perfusion apparatus described in this report measures volumetrically the oxygen uptake of the perfused organ by recirculating the gas mixture in a closed system. It is a modification of perfusion equipment previously described.^{5, 7} The apparatus is enclosed in a thermoregulated cabinet.* The system consists essentially of three components: A pump which maintains blood flow; a "lung" which ventilates the blood and an organ which utilizes this blood (fig. 1).

The circuit of the perfusion fluid is conveniently traced beginning with the reservoir flask from which the perfusate is lifted through a filter to remove any clots that may form during the experiment. The pump, a Brewer pipetting machine, with variable speed and stroke, activates a finger-stall pump. Uni-directional flow is obtained by attaching glass perfusion valves to the finger-stall pump. From this point blood passes to the top of a condenser column that functions as an artificial lung. The blood is spilled from the top of the "lung" as a thin, falling film passing counter-current to a stream of oxygen which oxygenates the blood and sweeps it free of its load of carbon dioxide. The "arterial" blood is collected in a small reservoir at the base of the "lung" where two outlets are provided, one channeling blood to the portal vein cannula, and the other serving as an overflow returning surplus blood to the reservoir flask. This overflow outlet is suspended 20 cm. above the liver, thereby providing a constant perfusion pressure. Oxygenated blood,

* Fabricated by General Service Division, Carpenter Shop, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington 12, D.C.

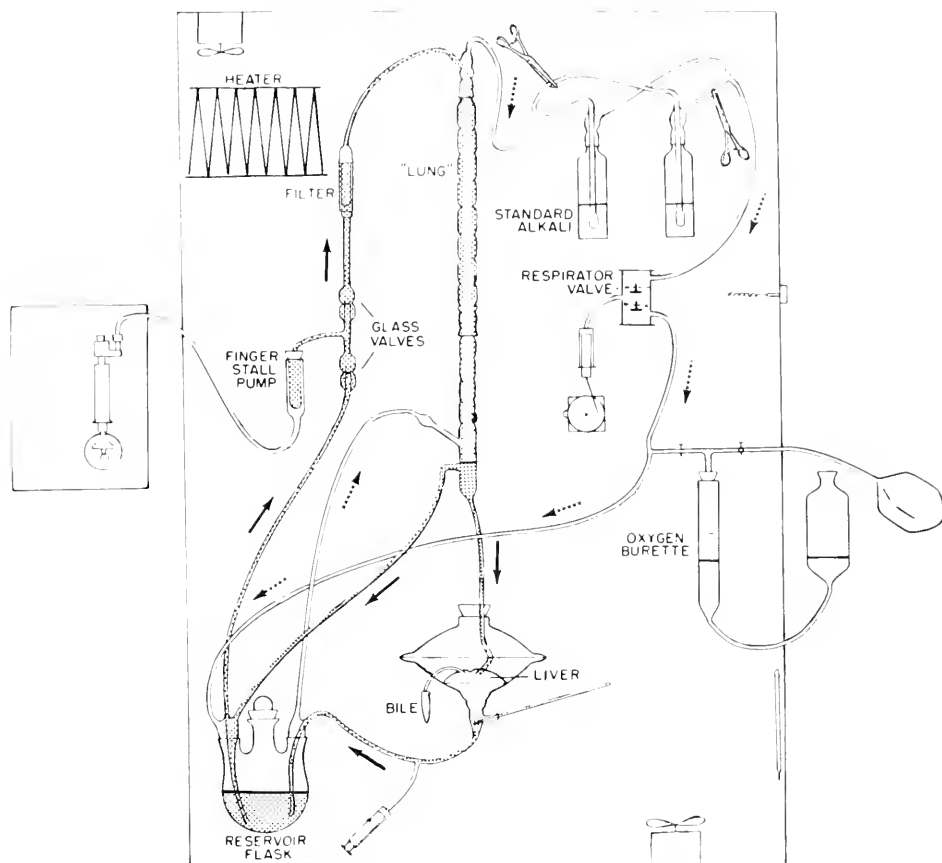


FIG. 1.—Schematic diagram of liver perfusion apparatus.

entering the portal vein, perfuses the liver. Hepatic vein outflow enters a thermometer well, thus affording an opportunity to observe the temperature of blood leaving the liver. Flow continues from the well through a flowmeter[†] and into the reservoir flask.

The ventilating mechanism is a closed circle absorption circuit system. Unidirectional gas flow is provided by a non-breathing type valve, the center chamber of which is attached to an activating pump, the "respiratory muscle" of the system. The pump[‡] is simply a 50 ml. syringe attached to a 60 r.p.m. motor by an eccentric wheel. From the respirator valve, gas flows through the reservoir flask carrying away any CO₂ that may have accumulated. Next, the gas enters the base of the condenser column (lung) just above the blood overflow opening. The oxygen-rich mixture passes up the column, oxygenating the thin film of perfusate falling down the internal surface of the "lung" and sweeping it free of its CO₂. The gas now

[†] A T-tube is inserted in the line and a 100 ml. syringe attached to the side arm. Flow rate is checked by drawing the venous outflow into the syringe while maintaining a constant blood level in thermometer well.

[‡] Fabricated by the Instrumentation Division, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington 12, D.C.

passes through the CO₂ absorber and the circuit is completed by returning the gas to the respirator valve and the base of the "lung." A graduated oxygen burette with leveling bulb is connected to the system.

Since all parts of the gas system are relatively rigid (except the water level in the oxygen burette and the blood level in the reservoir flask, including the blood-filled tubing leading to the thermometer well) the oxygen consumption is read directly from the graduated burette, after restoring the initial manometric pressure (1 cm. H₂O) with leveling bulb. It is essential that the level of blood in the thermometer well be at its initial level each time an oxygen reading is made. Since changes in the volume of fluid within this rigid-walled system will be reflected in the oxygen measurements, appropriate corrections must be made for the volume of all samples withdrawn or materials added to the system. Another correction factor is required when the standard alkali bottles are changed. The final volume of oxygen utilized is then corrected to standard temperature and pressure.

The liver preparation with portal vein and bile cannulae in place is supported on a wire-mesh stage wrapped with cellophane to protect the liver. The stage and liver are enclosed between two glass dessicator lids. This minimizes the loss of CO₂ diffusing from the liver capsule and hepatic vein outflow, and maintains the liver in an environment of constant humidity. The opening in the upper lid contains a rubber stopper with glass tube connecting the "arterial" flow from the "lung" with the portal vein cannula, while the opening in the lower lid has a standard taper joint fitted to the thermometer well. The bile cannula is placed through a small opening drilled in the lower lid and a graduated tube attached to collect bile.

Methods. A non-fasting rabbit of either sex is anesthetized with pentobarbital (30 mg./Kg.) and heparinized. The common bile duct and portal vein are cannulated. The liver with attached cannulae is rapidly excised, weighed, and placed in the perfusion apparatus. The liver is without circulation for an interval of 5 to 10 minutes. The perfusate used is heparinized whole rabbit blood drawn from donor rabbits by cardiac puncture. The pooled donor blood (300 ml.) is diluted with normal saline (150 ml.) and a commercial 5 per cent protein hydrolysate solution in 5 per cent glucose (50 ml.). The final hematocrit is 20. Aureomycin, 25 mg., is added to the perfusate. The gas system is flushed with oxygen several times, and an equilibration period of 10 to 30 minutes allowed for the gas mixture, perfusate, and liver before starting measured observations.

Oxygen uptake was determined volumetrically using the method described above. Carbon dioxide production was determined by titration of the standard alkali solution.

When the conjugation of morphine or thiopental was to be studied, 50 mg. of the drug were added to the reservoir flask and hepatic vein samples analyzed for free and bound morphine.

When the effect of temperature was to be studied, the temperature of the blood and cabinet for the first 90 minutes was 24° C.; then the cabinet heater was started and the temperature raised to 37° C. for two hours, a second dose of 50 mg. morphine was added to the reservoir and hepatic vein samples analyzed.

Free and bound morphine present was determined by the ultraviolet spectrophotometric method of Goldbaum.⁸

In other experiments (to study the effect of thiopental on morphine conjugation), 50 mg. of thiopental were added to the reservoir flask, "arterial" and "venous" samples drawn and analyzed by the method of Jailer and Goldbaum.⁹ Morphine sulfate (50 mg.) was added to the perfusate at the same time as thiopental, and both "arterial" and "venous" samples followed for free morphine content.

A graduated test tube was attached to the bile cannula and the volume collected was recorded at hourly intervals.

Results and discussion. A one-hour control period was observed in all experiments before either morphine or thiopental was added to the perfusate. Thus, each liver served as its own control concerning the effect, if any, of the drugs on oxygen uptake and carbon dioxide production. With the concentrations used, neither morphine nor thiopental caused observable changes in oxygen uptake or carbon dioxide production.

Figure 2 and table I summarize the effect of temperature on liver respiration. The marked depression in respiration as a result of the decreased temperature is quite apparent. However, regardless of the temperature, the oxygen utilization and carbon dioxide production remains linear over the time followed.

The significantly ($p < 0.025$) low RQ value of the liver during the first hour of perfusion at 37° C. was unexpected (table II). This might be explained as being

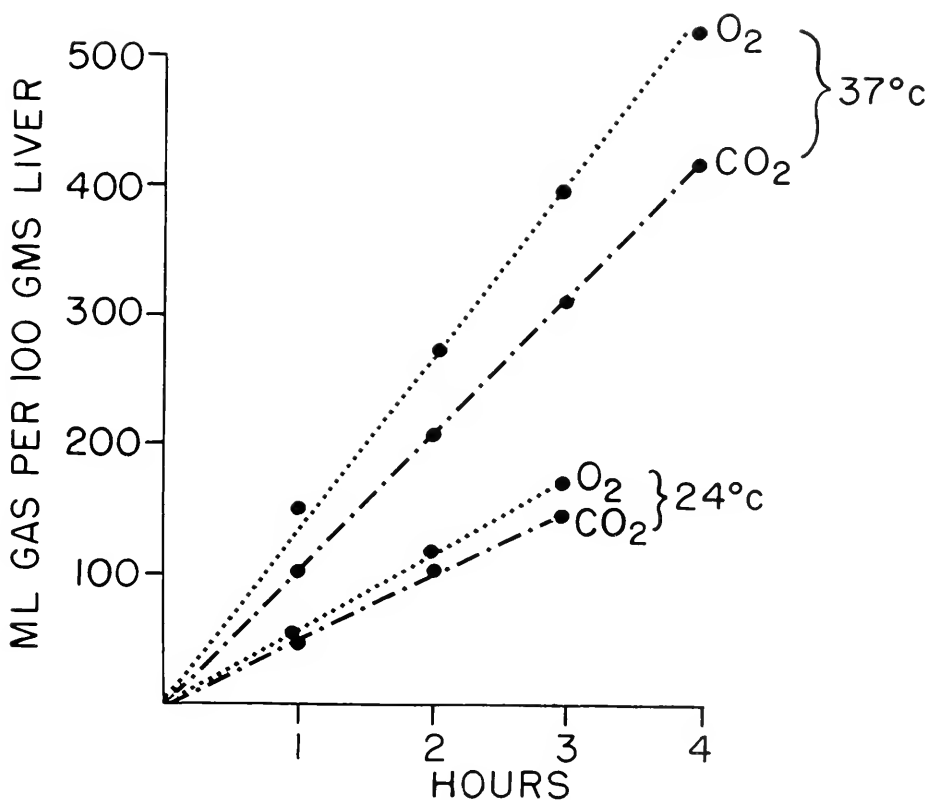


FIG. 2.—Effect of temperature on liver respiration.

TABLE I

EFFECT OF HYPOTHERMIA ON LIVER RESPIRATION

Temperature	Oxygen consumption ml./hr./100 gm.				Carbon dioxide production ml./hr./100 gm.			
	Time after perfusion started, hrs.							
	1	2	3	4	1	2	3	4
37° C.	162±4	140±11	133±9	131±10	111±8	113±8	120±10	110±8
24° C.	68±10	63±7	55±7	47±8	59±7	54±6	44±2	39±3

All values ± standard deviation of the mean.

TABLE II

EFFECT OF HYPOTHERMIA ON THE RQ OF THE ISOLATED PERFUSED LIVER

Temperature	RQ			
	Time after perfusion started, hrs.			
	1	2	3	4
37° C.	0.70±.04	0.83±.04 ^a	0.89±.09	0.84±.08
24° C.	0.85±.09 ^a	0.87±.12	0.80±.10	0.85±.15

All values ± standard deviation of mean.

^a P < 0.025 when compared to 1 hr. RQ at 37° C.

the result of the demand for energy at 37° C. being greater than that at 24° C. and consequently the liver, being unable to metabolize carbohydrate efficiently, burns fat in an attempt to supply the necessary calories. However, at 24° C. the liver is capable of meeting the limited energy demands by oxidizing an amount of glucose sufficient for its needs. Apparently the liver is soon capable of utilizing the available carbohydrate more efficiently and in the second hour of perfusion at 37° C., the RQ returns to normal.

There is a marked effect of hypothermia as well as that of barbiturate on the ability of liver to detoxify morphine. The biologic half-life for the loss of free morphine from the plasma at 37° C. is 3.7 minutes; 94 minutes at 24° C.; in the presence of pentothal at 37° C., 14.7 minutes. Table III summarizes these figures and, in addition, gives the biologic half-life of thiopental.

Figure 3 illustrates the effect of hypothermia on morphine conjugation. Figure 4 is the portion of the curve from 1–2 hours and shows that following the injection of morphine at 24° C., the maximum value of free morphine is not reached for approximately 12 minutes and falls off quite slowly. Figure 5 is that portion of the figure from 5–6 hours and shows that following the injection of morphine at 37° C., the maximum concentration in the perfusion fluid is reached in 3 minutes and falls

TABLE III

EFFECT OF HYPOTHERMIA ON DRUG DETOXIFICATION BY LIVER

	No. of Exp.		Half-Life (Minutes)	
	37° C.	24° C.	37° C.	24° C.
Morphine	8	4	3.7	94 ^b
Morphine (with pentothal)	4	—	14.7 ^a	—
Pentothal	5	2	46	530
				185

^a P < 0.05 when compared to morphine alone.

^b P < 0.001 when compared to morphine at 37° C.

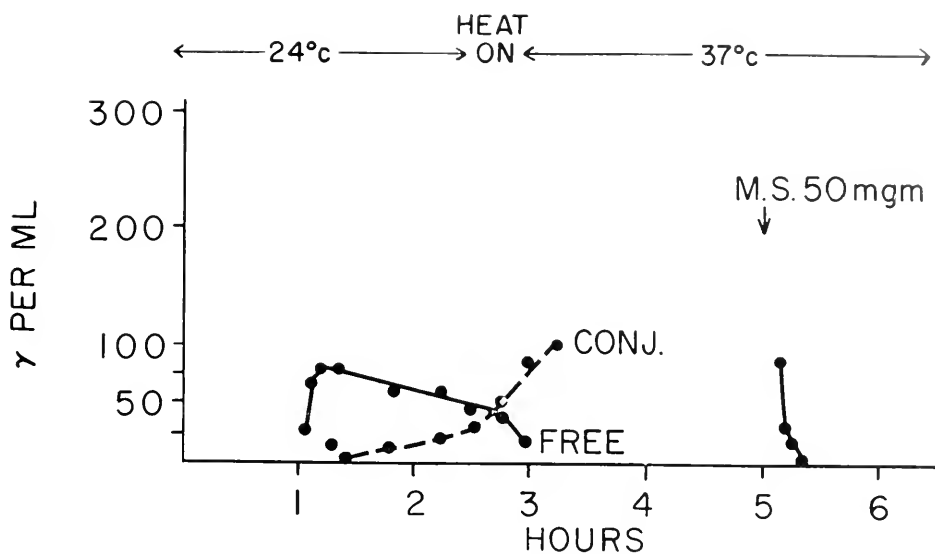


FIG. 3.—Effect of temperature on morphine metabolism by perfused liver.

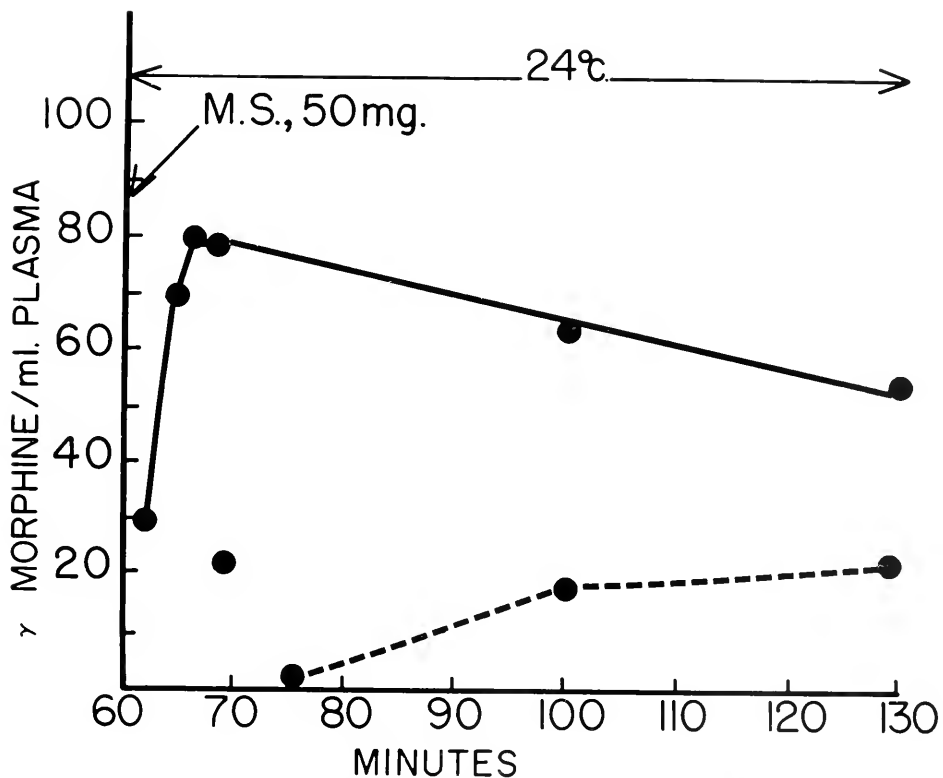


FIG. 4.—Morphine metabolism by perfused liver at 24 C.

off very rapidly. These measurements were made at the venous outflow of the liver.

Whereas the level of free morphine rises extremely rapidly in the hepatic outflow at 37° C., there is a very definite slowing of the mixing time at 24° C. as illustrated in figures 4 and 5. This may well be the result of the flow of the perfusion fluid which was decreased by about 25% but with a large variation at both temperatures.

There was a three to six minute delay in the appearance of the bound morphine suggesting that morphine is held briefly by the hepatic cells while being conjugated. Further support of this possibility was observed by the rise in level of bound morphine in the hepatic veinblood for 10 to 15 minutes after all free morphine had been removed from the "arterial" blood.

The rate of bile formation at 37° C. was two to four times that observed at 24° C.

These observed metabolic effects may well be the result of the inability of the liver to form the glucuronide of morphine.¹⁰ It has been recently demonstrated^{11, 12} that the glucuronide is formed through the formation of uridine diphosphate glucose. In view of the markedly reduced respiration of the liver as a result of the hypothermia, it is not unexpected that this mechanism is slowed down with a

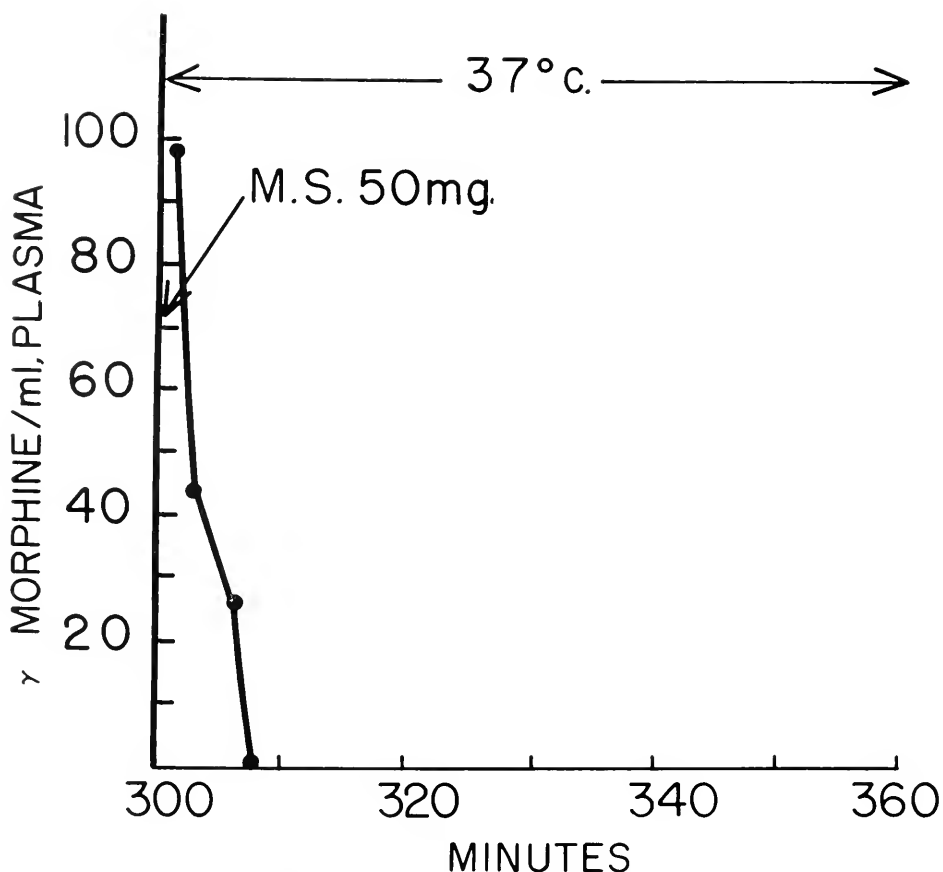


FIG. 5.—Morphine metabolism by perfused liver at 37° C.

corresponding decrease in the binding of morphine. Furthermore, in light of the reported fact that thiopental uncouples oxidative phosphorylation^{13, 14} and generally reduces cellular respiration, we have another mechanism for slowing the conjugation system of morphine.

In a similar manner, the increase in the biologic half-life of the thiopental at 24° C. may also be the result of the decrease in the respiration of the liver and the inability of the organ to carry out its oxidative processes, normally. It has been shown for dialkylbarbiturates,¹⁵ for thiobarbiturates¹⁶ and barbiturates generally¹⁷ that the detoxification is brought about by oxidative processes mainly in the liver.

The effect of the temperature change on the urea production by the liver is also quite marked. The average urea N production at 37° C. was 11.48 ± 0.88 mg./hr./100 gm. liver while at 24° C. it was 7.52 ± 0.92 mg./hr./100 gm. liver. These data were significant at the 2.5% level using the "t" test. MacKenzie and du Vigneaud¹⁸ have shown that the carbon for urea formation arises from respiratory CO₂. Ratner and Pappas¹⁹ demonstrated that the "ornithine cycle" required ATP and a mechanism for maintaining the level of ATP, i.e., the oxidative citric acid cycle. In view of decreased respiration resulting in decreased CO₂ production and oxidative metabolism, the observed decrease in urea production is not surprising.

Summary. A liver perfusion apparatus with closed gas system providing a means of measuring oxygen uptake volumetrically has been described. The effect of hypothermia on oxygen uptake, carbon dioxide production, morphine and thiopental detoxification, and bile formation in the isolated perfused rabbit liver was observed. Also, the effect of thiopental on morphine conjugation was followed. It is believed that the alterations in metabolic activity observed *in vitro* with liver perfusion can be attributed to the effect on respiration and oxidative phosphorylation.

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THE EFFECT OF HYPOTHERMIA ON THE ISOLATED PERFUSED RAT LIVER*

RALPH W. BRAUER

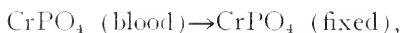
The balance of processes essential to maintenance of life in a homeotherm depends upon a complex interplay of chemical and physical factors. Cooling the tissues of such organisms would be expected to lead to a multiple dislocation of components of this balance and should be compatible with survival only under rather special circumstances. As an example of a highly polyfunctional tissue with a relatively simple physical framework the liver should lend itself particularly well to the exploration of these relations. Development of techniques which allow maintaining the isolated rat liver outside the body as a functioning organ for well over a day¹ allows us to study the effects of hypothermia on this mammalian organ in the absence of the circulatory and regulatory complications likely to ensue when chilling a whole animal. I should like to report here on results obtained to date in this field, and to discuss their bearing upon problems of the physiology of the hypothermic animal.

Of the physical parameters which determine liver function, the hemodynamics of this organ are the most readily accessible. Elsewhere² we have shown that the flow pressure diagram of the isolated rat liver perfused through the portal vein can be thought of as consisting of three parts: a region in which perfusion pressures determine the number of open channels (opening pressure region), a region in which limited dilation of otherwise open channels determines the flow pressure diagram, and finally at pressures slightly above the physiological range a region where flow is directly proportional to pressure and the liver vasculature behaves as a rigid system of conduits. Lowering the perfusion temperature results in increased resistance to blood flow through the liver. However, if the flow coordinate is scaled so as to superpose the curves in the region of direct proportionality, the remainder of the flow pressure diagrams also are found to superpose upon each other at perfusion temperatures between 17 and 38° C. at least. This I take to imply that neither the opening pressures nor the dilatation of the vessels of the isolated liver are affected by cooling; the flow pressure relations would appear to reflect a change in perfusate viscosity exclusively. Thus, for any given pressure in the rigid conduit region, liver blood flow changes with temperature in the fashion shown in figure 1. The results yield a straight line on Arrhenius coordinates between 17 and 38° C. and from the slope of this line an activation energy of 6570 cal. can be calculated for this process. Plotting in the same manner the fluidity of blood (the inverse of viscosity) as measured in an Ostwald viscometer³ a similar line is obtained, the molar activation energy this time being about 6400 cal.—substantially identical with the above value for the isolated liver.

I do not yet have any data on the second major group of physical variables affecting liver function, those having to do with subdivision of the organ into dif-

* The opinions or assertions contained herein are those of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Establishment at large.

ferent distribution spaces (or fluid compartments, if you prefer). Going on, however, to transfer problems, I do have information on what I believe is the simplest transfer process in the liver, the irreversible removal from the blood of an inert radiocolloid by the reticuloendothelial cells of the liver. We have studied P^{32} -labelled $CrPO_4$ colloid,^{4, 5} and have shown that three factors determine the rate at which this process occurs: the mean transit time of blood through the liver, the surface to volume ratios of the hepatic blood channels, and the basic rate of the uptake reaction



or, if you prefer, the probability of the micelles' sticking to the surface of a Kupfer's cell when they come in contact with one. Reducing the perfusion temperature on the whole tends to lengthen transit time at any given perfusion pressure, as you will readily perceive if you consider what we discussed in relation to hemodynamics. Since I do not know yet what changes in liver blood volume result from cooling, I cannot discuss the surface to volume ratios beyond venturing an opinion that changes due to the temperature here are likely to be very small. Thus, any major changes in $CrPO_4$ extraction due to cooling should reflect primarily a modification of the rate of the basic reaction. In fact, lowering the perfusion temperature markedly decreases the efficiency of $CrPO_4$ uptake, especially at perfusion rates above the physiological value of 1 cc./g./minute. The dependence of extraction efficiency upon temperature at a constant perfusion rate is shown in figure 1, the results indicating a molar activation energy of 15,400 cal. for this process. While data are not yet complete regarding the dependence of extraction efficiency upon perfusion rate at various temperatures, results to date are in accord with the hemodynamic picture given above.

Figure 2 illustrates, by means of a typical protocol, some of the other changes in liver function resulting from lowering of perfusion temperatures below the normal 38° C. Blood glucose levels are reduced under those conditions, although the resultant level is not a monotonous function of temperature: the blood glucose levels attained at 30° invariably have been found lower in the isolated liver preparation, than those reached at 25° C., as well as being lower than those obtaining at 38° C. Concerning the mechanism of this response little can be said at the moment beyond the fact that it is apparently fully reversible. Glycogen concentrations in preparations cooled and rewarmed do not differ significantly from those found in preparations maintained throughout at 38° C. We are planning experiments with C^{14} glucose to establish whether the fall of blood glucose levels reflects increased glycogen deposition, decreased glycogenesis, or an unlikely increase of glucose utilization at the lower perfusion temperatures.

Bile flow decreases dramatically with cooling. While there is some variation from preparation to preparation, the mean relative decrease of bile flow with perfusion temperature once again obeys an equation of the Arrhenius type over the range from 17 to 38° C. Calculated activation energy here is 33,900 cal. This value is close to that obtained for our strain of rats *in vivo*, using surface cooling under sodium pentobarbital; for another strain of rats, however, Kalow⁶ reported values yielding an Arrhenius energy closer to 15,000 cal. It remains to be established

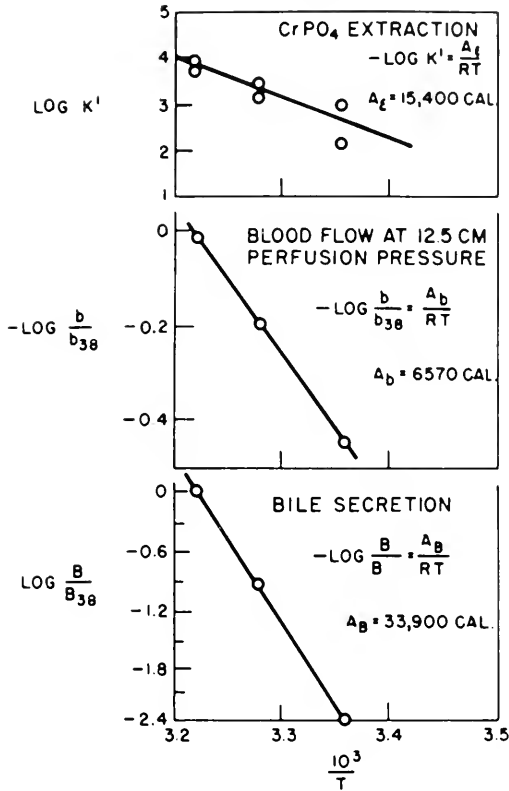


FIG. 1.—Temperature coefficients of CrPO_4 extraction (k' = reaction rate constant \times active surface concentration), blood flow rate at 12.5 cm. perfusion pressure, and bile secretion rate.

whether these values reflect some peculiarity of the urethane anesthesia employed by Dr. Kalow, or whether the discrepancy reflects real strain differences in the temperature dependence of this function.

Recovery of bile flow from hypothermia is usually prompt in livers cooled to about 30° as well as those cooled to 17°C . At temperatures lying between these limits, however, our experience has shown a relatively high proportion of failures of bile flow to recover on rewarming. Since somewhat parallel observations were made in connection with the colloid uptake studies, and perhaps may also underlie the glucose picture, one might give thought to the possibility of a critical temperature range in which the rat liver may suffer most severely from cooling. In figure 2 it should also be noted that on rewarming after a period of hypothermia there is a slight overshooting of bile flow rate, lasting perhaps 20 minutes before the new plateau of bile flow is reached at a level close to that seen before cooling.

During the periods of hypothermia bile composition does not appear greatly changed. Bile solids remain constant, and our short series of cholate determinations suggest no significant drop in the concentration of bile salts in bile. The BSP data, to be discussed presently, also indicate normal concentrations of this dye in bile.

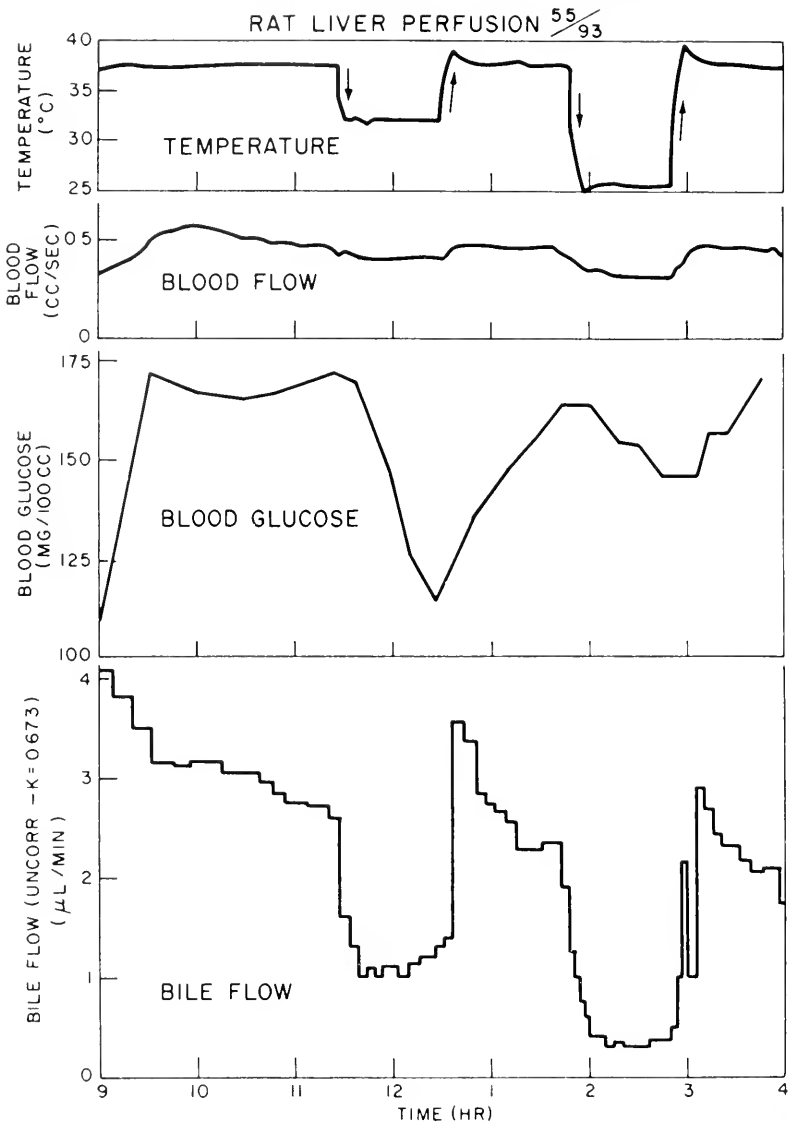


FIG. 2.—Protocol of liver perfusion experiment 55-93, showing effects of cooling to 32° C. and to 25° C. upon blood flow, blood glucose, and bile flow.

The hypothermic rat liver is capable of responding to choleric stimuli at 30° C., and in general also at 25° C.⁶ The relative increase in bile flow following dehydrocholate at 25 to 30° C. is as great as that at 38° C., though the absolute response—measured as extra bile volume—is, of course, reduced in proportion to the basal bile flow. At 25° C. or lower we have at times seen a curious phenomenon which we have dubbed the “freezing in” of the choleric effect. In such cases there will be a minimal choleric response at the low temperature but a very marked overshooting of bile flow rate on rewarming. Since this response is probably related to

retention of the choleretic at the effector sites, this response suggests that the liver in some respects acts as though it were subjected to bile stasis; this response is not too different from that seen if the organ were subjected to mechanical bile stasis. The hypothermic liver, therefore, may be thought of as in "biochemical stasis" under these conditions. We shall see presently that this concept also describes parts of the BSP handling in the liver at low perfusion temperatures.

Our interest in hypothermia originally sprang from a concern with the mechanism of bile secretion. The positive correlation of bile flow and perfusion temperatures under controlled conditions of blood flow clearly separated this secretion from urine—a filtration reabsorption product with a negative correlation with temperature under comparable conditions.⁷ As a further outcome of our concern with this problem we studied the relation between bile flow, perfusion rate or pressure and perfusate oxygen tensions. Figure 3 shows our results insofar as they relate to the problem of hypothermia. It is evident that, while at 38° C. bile flow becomes dependent upon perfusion rate when this falls below 2 cc./g./min., at 29° C. much lower perfusion rates can be attained without significant falling off of bile flow. From our point of view this finding is in line with the results of pressure chamber and of hemodynamic studies indicating that oxygen supply in relation to oxygen consumption, rather than perfusion pressure or perfusate flow, is the factor limiting bile flow under these conditions. From the point of view of the applications of hypothermia to surgery or medicine, these same data afford a further illustration of the protection of tissue functions against ischemia by lowering of tissue temperatures.

Finally, a few words may be in order in relation to the handling of BSP by the isolated rat liver at various temperatures. After injection, BSP disappears from the circulation according to a time course which is exponential for a brief period,

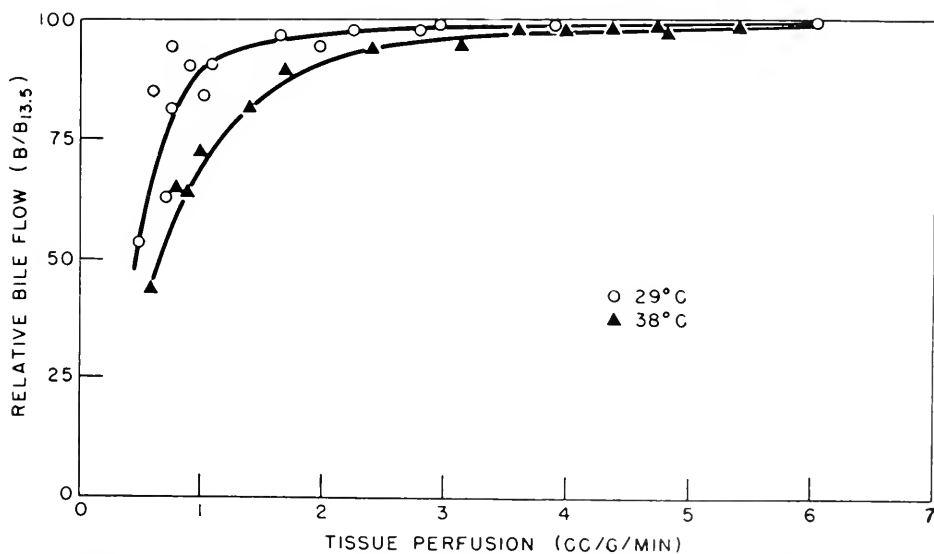


FIG. 3.—Bile flow-blood flow relations at 38° C. and at 29° C. for three isolated rat liver preparations. Whole blood used as perfusate.

slowing down gradually as the dye accumulates in the liver. At temperatures below 38° C. two changes supervene: the efficiency of extraction at the start decreases and the exponential portion of the disappearance curve is shortened, extraction efficiency falling off after extraction of a much smaller fraction of the injected dose. Excretion of dye in the liver into bile apparently is affected only to the degree that bile flow is delayed. As shown in figure 4, peak concentrations of BSP (or of its derivatives) in the bile are unaffected by cooling to 29° C. at least. Figure 5 finally shows that initial BSP extraction efficiencies are far less affected by cooling the organ than is the bile flow or, by implication, the rate of re-excretion of the dye into bile. While a discussion of these results (which have important bearing on the problem of BSP transfer mechanisms, and the coupling of uptake and excretion of this dye) is beyond the scope of this communication,⁸ it seems proper to point out that once again the changes in liver function in hypothermia resemble remarkably those seen in subtotal bile stasis.

Looking back over what has been reported here, the effects of hypothermia on liver physiology seem to fall into three categories: circulatory effects, dominated by the change of blood viscosity with temperature; certain functions which fall off in accordance with the classical Arrhenius relation, and with varying activation energies (bile flow, CrPO₄ colloid uptake); and, finally, certain complex effects manifesting imbalance of various metabolic chains in the hypothermic organ. Examples of this last group are the equilibrium levels of blood glucose, the indication of a critical zone for non-recovery of certain functions, and the phenomena tentatively grouped together as reflecting hypothermic bile stasis: the overshooting of bile flow, "freezing in" of choleresis, BSP uptake curves. It is our feeling that exploration in this third group of phenomena is most likely to lead to answers to the problems of interest to us all in relation to the survival and recovery of the homeothermic organism subjected to hypothermia.

From another point of view, the relations between blood flow and bile flow in the hypothermic preparation (figure 3) are an illustration of the protection afforded against ischemia by hypothermia, and to those of us interested in liver physiology may suggest a number of extension experiments in relation to the action of noxious agents upon this organ and their possible counteraction by regional hypothermia.

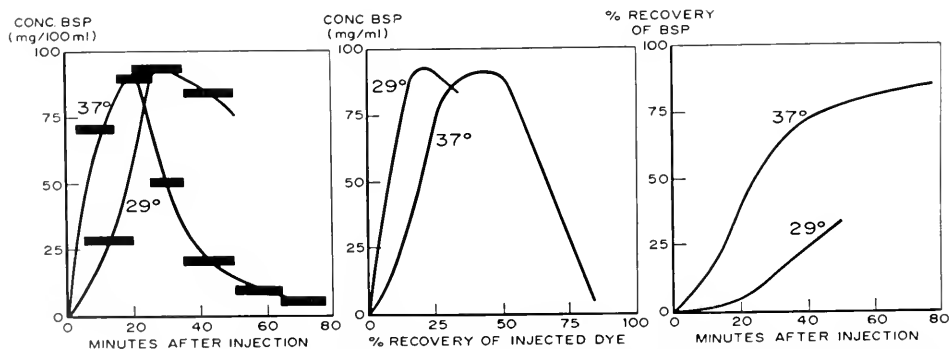


FIG. 4.—Relations between biliary BSP excretion, time, and per cent recovery of injected dose, following injection of two doses of 5 mg. BSP each, one at 38° C. and one at 29° C. per fusion temperature.

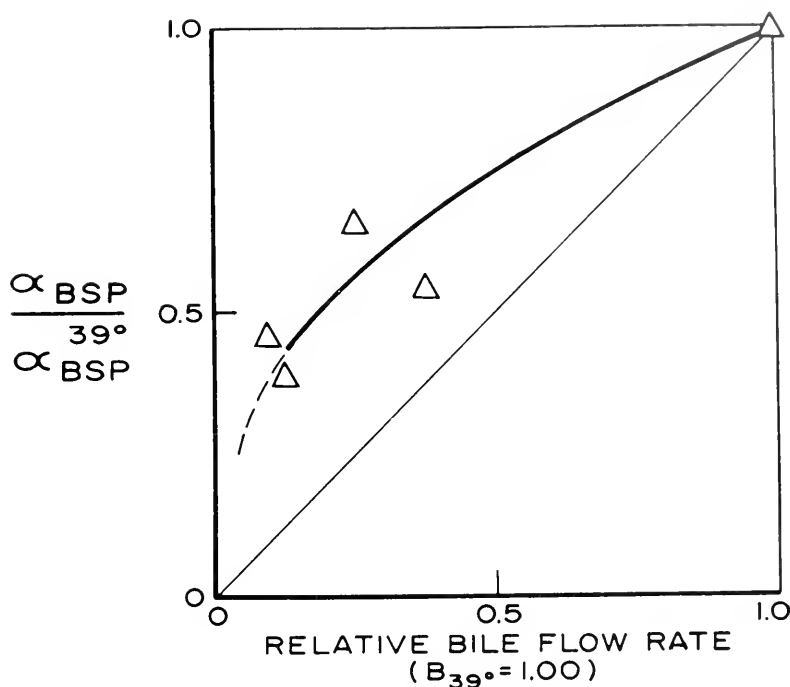


FIG. 5.—Relation between bile flow and BSP extraction efficiency (as fraction of BSP in blood removed by liver in one passage) at temperatures below 39° C.

Summary. 1. The effects of hypothermia upon the functioning of the isolated perfused rat liver have been studied.

2. Hemodynamic changes due to cooling of this preparation appear to be confined substantially to consequences of the increased blood viscosity.

3. Chromic phosphate colloid uptake by the liver is slowed by cooling. The relations appear to fit the Arrhenius equations with an activation energy of 15,400 cal.

4. Cooling results in lowering of perfusate glucose levels relative to those at 38° C. There is evidence of a minimum around 32° C.

5. Bile flow is slowed in the hypothermic liver. Between 38° C. and 17° C. the values can be described by the Arrhenius equation with an activation energy of 33,900 cal. At 30–32°, and at 17° C. this fall of bile flow is generally reversible, while at 25° C. a significant fraction of non-recovering preparations were seen. Some detailed changes in bile flow recovery, choleresis, and bile composition have been presented. Decreased dependence of bile flow upon blood flow at slow perfusion rates has been demonstrated in the hypothermic liver.

6. The effect of hypothermia upon uptake and excretion of sodium sulfobromophthalein disulfonate (BSP) by the liver has been discussed. Changes consist in some slowing of initial dye extraction, premature evidence of saturation of the uptake mechanism, normal bile BSP concentrations but delayed excretion of the dye due to retardation of bile flow.

7. These results have been discussed from the point of view of their bearing upon the clinical applications of hypothermia and upon the physiology of the hypothermic homeotherm.

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EFFECTS OF COLD ON THE NERVOUS SYSTEM

J. D. McQUEEN

I would like first to outline in a general way the development of our knowledge of the effects of induced cold on the functioning of central and peripheral nervous tissues, and second to consider the specific effects of peripheral nerve and cerebral cortex.

Simpson^{27, 28} stands virtually alone as the pioneer in this field; however, prior to his work, Walther¹¹ in 1862 had cooled the rabbit to a temperature of 20° C. with successful rewarming, and Horvath¹⁹ in 1881 similarly studied the hibernator to a temperature just above 0° C. Horvath, as well, did, during the period of hypothermia, elicit muscular response upon stimulation of the appropriate motor nerve.

Sutherland Simpson in 1902²⁷ was able to cool one monkey to a rectal temperature of 14° C. He rewarmed this animal and examined it thereafter for a period of one month. His description reads, "There was no evidence whatever of any bad effect having followed." With Herring in 1905²⁸ he pursued this work in the cat and established a lethal temperature of 16° C. for this animal. He introduced both terms "cold narcosis" and "artificial hibernation" at this time, the connotation of the latter term being quite distinct from that introduced more recently by Laborit and his co-workers.²³ He found that, at a temperature estimated to be between 23° and 25° C. for both animals, the heat regulating mechanisms were completely suppressed. Simpson thus rather early defined the narcotic action of cold. He perhaps correctly localized such action in the brain stem, for the term "highest centers" does appear in his description. Britton,⁵ some two decades later contested this concept of artificial hibernation on the basis of Simpson's brief periods of observation. He did show, that after periods of 6 to 12 hours under favorable conditions, the animal could restore heat regulation. Both workers considered in detail during the rewarming period, the temperature course of recovery from neurologic deficit. These are listed in table I.

Not until 1940 was general refrigeration employed in the human. Temple Fay¹⁰ first employed this technique and did so specifically in the treatment of advanced malignancy. Forty-two patients were so treated to a minimum temperature of 25° C. and for periods as long as 150 consecutive hours. Abiding neurological deficits were not observed, nor did subsequent microscopic examination of these brains by Sano and Smith²⁵ reveal significant pathology. Talbott²⁹ reported on a group of similarly treated psychotic patients in 1941, and Laufman in 1951²⁴ presented the best documented case of accidental hypothermia. From these cases, the neurological status at the various recorded temperatures can be tabulated, although the detail has never been comparable to that of the animal experiments.

It is recognized that no rigid temperature level exists for disappearance of a response on cooling nor reappearance of the same response on rewarming. Unfortunately, the temperatures recorded were rectal and not tissue temperatures, and non-volatile anaesthetic agents were routinely used. In the human at a temperature at about 34° C. amnesia is established for the period of cooling below this level.

TABLE I

MINIMAL TEMPERATURES (RECTAL) FOR SELECTED REFLEXES AND OTHER REACTIONS AS OBSERVED IN THE REWARMING PERIOD OF THE CAT

(From Simpson and Britton)

Knee jerk	persisted to 16° C.
Respiratory reflex to pain.....	persisted to 16° C.
Ankle jerk	recovered at 17° C.
Flexor withdrawal	recovered at 20° C.
Spontaneous stepping motion.....	recovered at 20° C.
Micturition reflex to pain.....	recovered at 16–24° C.
Phonation to pain.....	recovered at 20° C.
Pinna reflexes	recovered at 20° C.
Clawing	recovered at 21.5° C.
Blinking reflex	recovered at 21.5° C.
Corneal reflex	recovered at 22° C.
Light reflex	recovered at 22–24° C.
Phonation, spontaneous	recovered at 22° C.
Swallowing and sneezing.....	recovered at 22° C.
Licking	recovered at 25° C.
Vomiting	recovered at 26° C.
Audition	recovered at 27.5° C.
Vision	recovered at 27–28° C.
Olfaction	recovered at 27.5° C.
Voluntary movements	recovered at 26–27° C.
Coordinated movements	recovered at 30° C.

The patient becomes dysarthric in this range and begins to lose contact with his surroundings, although pain is readily appreciated. However, in the 30° C. range some patients can recognize relatives and other persons familiar to them. At about 27° C. they are unable to respond to verbal stimuli and voluntary motion is lost. The pupillary light reaction, deep tendon reflexes, superficial skin responses and the gag reflex are lost at a level of 25° to 26° C. Because of the limitation imposed by cardiac dysrhythmias at lower temperatures on homeothermic animals, the cerebral response to cooling in the range 8°–18° C. was not readily appreciated until the work of Jensen and Parkins.²⁰ They differentially cooled the brain to temperatures of 8°–12° C. in nine dogs, noting gross neurological damage in seven dogs and fatal cardiac arrhythmias in two. However, in seven dogs cooled only to the range of brain temperatures of 12°–18° C., neurological abnormalities were not observed.

In summary, above the common level for ventricular fibrillation, Simpson demonstrated the absence of gross damage to the central nervous system and the presence of a state of narcosis for at least a period of several hours. Below this level, Jensen and Parkins have found definite and presumably irreversible injury. Unfortunately the specific temperatures and exposure times for such injury have not been determined.

Peripheral nerve. Forbes and Ray, in their classical paper in 1923,¹¹ established the preservation of excised mammalian nerve function to a level of about 8° C. They showed that the survival period of these nerves varied inversely with the temperature, and thus provided a routine laboratory method of nerve preservation.

They indeed provided the first direct evidence that nervous tissue can better tolerate hypoxia when cooled. Chatfield *et al.*⁷ demonstrated a species difference in comparing the responses from the cooled tibial nerves of the rat and hamster. The critical temperature for the former was 9° C. and for the hibernator 3.4° C. This latter level is just below the body temperature maintained by the animal in deep hibernation. They further showed that the nerves from hibernating and cooled non-hibernating hamsters reacted identically.

Kahana, Rosenblith, and Galambos²² noted the neural component of the round-window response of the hamster to be absent below a temperature of 18° C. They thus established a specificity amongst nerves of the same animal.

Gasser¹⁸ found the amplitude of the action potential of frog peripheral nerve to be decreased on cooling. The temperature coefficients for conduction time, spike duration and refractory phases were quite distinct, but all were greatly augmented in the lower temperature range. Chatfield and his group⁷ confirmed these results for the golden hamster. The action potential height, conduction velocity and excitability all decreased in a linear fashion although at a slower rate in the hibernator.

Denny-Brown *et al.* in 1945⁹ studied the results of direct freezing of mammalian nerve, as others had done before. In addition, they enclosed nerves within small metal cuffs, through which cooling fluids were circulated. The nerve temperature was then brought to a level somewhat above freezing. They were so able to establish definite damage to both the myelene sheaths and axis cylinders of the largest fibers at levels of approximately 8° C. and for periods as short as 10–15 minutes. They found motor deficit to precede sensory loss, and the sensation of touch to be affected before that of pain.

From this and other work (Lundberg²⁹), it is known that C fibers in general are much more resistant to cooling than A fibers. With minimal lesions, the largest myelinated nerve fibers will be selectively involved. The effects of cold at the range of 8°–10° C. and perhaps higher are quite comparable to the effects of ischemia.²⁶ The minimal injury induced by cold may well explain in part at least those peripheral neuropathies now being observed during the course of general hypothermia in the human. It is noteworthy that sympathetic fibers are numbered amongst those most resistant to the action of cold.

Central nervous system. Electroencephalography has perhaps best monitored the narcotic action of cold on the brain. Records are available for dog,²⁵ cat,²⁵ rat,⁴⁰ various hibernators,⁸ monkey⁶ and man.³⁶ The patterns are generally uniform for the group, although a species difference is recognized within hibernators.³⁰ Pre-cooling potentials varying between 50 and 100 μ v are registered with the animals under light barbiturate anaesthesia. A decrease in amplitude is usually not noted until a range of temperatures between 32° and 36° C. is reached. The potentials from occipital and parietal regions fall at the upper limit of this range, those from the frontal areas at the lower limit. The voltages then drop evenly to disappear at the level of electrical silence which is at a rectal temperature of 18°–20° C. One noted exception to this general decline is the introduction of delta waves of large amplitude (100 μ v) found at the 30° level and predominantly in the frontal region. Changes in wave form and frequency also became first apparent in the 32°–36° range. An intensification of the delta and theta activity with a loss of intricacy of

pattern first in the beta and then in the alpha frequencies occurs. These slower rhythms, when present alone, usually present a synchrony which is both hemispheric and inter-hemispheric. The theta rhythms are lost at about the 25° C. level. Very slow delta waves thereafter gradually decline until the end. During the re-warming period this pattern is reversed and reproduced in detail. The frequently observed lag of 1°–2° C. is perhaps best explained on the site of temperature recording. No significant changes are noted with repeated or prolonged periods of cooling and subsequent electroencephalograms are consistently normal.

Gaenshirt *et al.*,¹⁷ in their study of the cat, described exponential relationships between temperature and frequency, as well as temperature and voltage, for the range of temperatures between 32° and 38° C. Two blocking mechanisms for enzyme systems were incriminated to explain the limits of this relationship. The term structural metabolism was used to denote the estimated 10–20% of normal brain metabolism found at and below the level of quiescence demonstrated with electrical recording.

Chatfield, Lyman, and Purpura⁸ found the electrocorticogram of the golden hamster during arousal to be quite comparable to that of the rewarmed non-hibernating hamster. They further showed that convulsive activity was not induced in the strychninized cortex of these animals below those temperatures at which spontaneous electrical activity appeared. Evoked cortical potentials from the sciatic nerve were noted with cortical temperatures as low as 9.1° C. and the cortex was further found to be electrically excitable down to a level of 12° C. (although the voltages employed were admittedly enormous). They thus demonstrated the functional integrity of the long tracts during cooling, and established the low resistance of the brain stem activating systems to cold.

Electroencephalographic techniques have further proved of value as an adjunct in the estimation of cerebral damage due to anoxia provoked under hypothermic states. Such techniques have been used extensively by Loughheed *et al.*,^{27, 28} Ripstein *et al.*,²⁹ Jensen and Parkins,²⁰ Scott,³⁶ and Gaenshirt *et al.*¹⁶ These followed the demonstration by Bigelow in 1950² of the survival of dogs held at 20° C. for 15 minute periods of circulatory occlusion. Scott³⁶ has recently found that, in the human, bilateral compression of cervical vessels at the 30° C. level or below produces no EEG change up to 8 minutes and only occasional delta wave preponderance to 12 minutes. Jensen and Parkins²⁰ noted similar protection for periods of 30 minutes in the dog with brain temperatures of 20° C. By comparison, the production of total cerebral ischemia in the normal adult cat at 37° C. results in a flat cortical record in some 15 seconds. Evoked cortical potentials are lost in less than 100 seconds. Gaenshirt, Schneider, *et al.*¹⁶ have recently employed an isolated cat's head preparation to estimate the periods of survival of cortical electrical activity with anoxia induced through a wide variation of temperatures. These survival times varied inversely as the temperature in an exponential fashion. They then measured the periods of recovery of this same electrical activity after one minute of anoxia. These times when plotted against temperature produce an unexpected curve which is parabolic in form. From 37°–31° C. these recovery periods vary directly with the temperature as would be anticipated. Below this level, an inverse relationship is indicated. The explanation of this finding is obscure—par-

ticularly because of the many variables introduced by this method of animal preparation. If this relationship exists in the intact animal, it may well indicate a rather precise range of temperatures for minimal cerebral damage during anoxia. This range, which forms the lower portion of their curve, is from 28°–33° C.

Fazekas and Hinwisch¹¹ were first able to prove directly the diminution of cerebral metabolism during general refrigeration. This was done in 9 dogs by establishing a fall in the arteriovenous oxygen difference in the presence of a reduced cerebral blood flow.

Field, Fuhrman, and Martin¹² later showed *in vitro* with the Warburg apparatus, that the cerebral oxygen consumption varied as a constant function of temperature. This same group¹³ further studied the rates of oxygen consumption of rat cerebral cortex slices measured at 37.7° C. following one hour at 0.2° C. and noted full recovery. These findings were confirmed *in vivo* by Rosomoff and Holaday¹⁴ in the dog. They found the cerebral blood flow and oxygen consumption to fall linearly and equally with the temperature. An estimated 6.7% decrease of blood flow per degree of temperature decline in the range 35°–25° C. was established. The minimal changes noted in arteriovenous oxygen differences is surprising.

Credit must go to Lougheed and Kahn²⁷ for establishing a method of prediction for the protection offered by cold to the brain threatened by ischemia. These predictions were made on the dog with a detailed analysis of the cerebral metabolic rate and confirmed by clinical evaluations, microscopic examination of the brains and by the analyses of lactate-pyruvate ratios and electroencephalographic data. They found that during the cooling, the total body oxygen consumption generally coincided with the cerebral metabolic rate. The fundamental work of Bigelow³ on oxygen consumption was thus extended to the brain. At 25° C. they noted the cerebral metabolic rate to be reduced to between 23 and 35% of control values. The rate at 30° C. is approximately 50%. They were successful in safely cooling 7 of 8 dogs to a temperature of 23.6°–25° C. with a 15-minute period of anoxia. One animal did show evidence of neurological damage. Further work by Lougheed, Sweet, White and Brewster²⁸ proved the value of this guide for neural survival periods in the human. In one of 2 cases studied, ischemia was tolerated for periods as long as 14 minutes 25 seconds (at 25.8° C.). This 15-minute period would appear to represent more than a maximal safe time limit. However, should it be halved (to 8 minutes at 26° C.), the brain would still be undamaged for a time more than double that permissible at normal temperatures.

Comparatively little attention has been directed toward study of the degree of protection afforded the spinal cord under conditions of hypoxia. Recently Beattie *et al.*¹ have evaluated such protection in normothermic and hypothermic dogs whose spinal cords were endangered by ischemia produced by a thoracic aortic occlusion. Hind-quarter paralysis was produced in 4 of 10 animals at rectal temperatures of 35°–29° C. with aortic clamping of 60 minutes. Ten dogs were cooled with the aorta clamped for 60 minutes and 5 similarly treated for 40 minutes. Questionable weakness was noted in one animal in the first group. Those of the second group displayed no weakness. Unfortunately, although the rectal temperatures are known to be below 30°, the specific temperatures for each occlusion were not given. However, cooling to a level much below 30° is indicated because of the high incidence

of ventricular fibrillation in other animals of this series. One can expect at normal body temperature an incidence of paralysis of 50% or more with the thoracic aorta clamped for one hour. These findings do, then, show this time limit to be over maximum even with cooling, and they do establish the basic prolongation of the survival period for the spinal cord during general hypothermia.

One other group of experimental data seems pertinent, and points directly to the innocuous character of limited hypothermia. Jeppson and Nielsen²¹ have recently found the number of lesions of the blood brain barrier induced with 50% Diodrast to decline sharply with a fall in temperature. In comparable groups of rabbits they found 8 lesions at 38° C., 5 lesions at 35° C., 4 lesions at 31° C., and 0 lesions at 25° C.

Mention should be made of several reports of one untoward effect. This is specifically a lowering of the threshold for the induction of cerebral seizures during cooling. Noell and his group³¹ first demonstrated this with recordings of the electrical activity of the cortex and striatum of the rabbit in 1952. They found parameters of electrical stimulation which were capable of producing widespread and prolonged convulsive activity over and beneath the cortex during cooling, but which were quite ineffective both before and after cooling. Similarly, normally subconvulsive amounts of metrazol were productive of seizure patterns during the period of hypothermia. The body temperatures for such change were not given in detail but were below 30° C. These findings were confirmed in the dog by Ferrari and Amantea¹² in the temperature range 22°–34° C. These authors detected patterns which they identified as generalized and petit mal in type and which were found to originate simultaneously in all cortical areas. The seizure patterns so evoked recall the work of Bremer in 1935⁴ on direct cooling of the cortex. He noted a rapidly reversible weakening of the oscillations with brief periods of local refrigeration. This depression phase persisted after the application of more intense and prolonged cold (ice) and was then followed with a phase of what was called intensification of cortical activity. Rewerts³² similarly on clinical grounds has indicted the exposure to cold as a causal agent in the production of epilepsy and other neurological abnormalities.

Finally, at least four current problems seem to demand attention. First, a determination of the exact range of brain temperatures for maximal safety during ischemia. Second, an assessment of the possibility of injury in the form of a lowering of the threshold for seizures or other change even at temperatures as high as 30° C. Third, a better understanding of the basic action of cold on the sympathetic nervous system; and lastly, an investigation of thermosensitivity of peripheral nerve during clinical hypothermia.

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EFFECTS OF CHANGES IN ARTERIAL $p\text{CO}_2$ ON CEREBRAL BLOOD FLOW AND METABOLISM DURING HYPOTHERMIA

JEROME KLEINERMAN

As Dr. McQueen has noted, there is evidence^{1,2} that the cerebral blood flow and cerebral oxygen consumption decrease proportionately with temperature at least to the levels studied, i.e., 25° C. The problem that is not yet clear is whether at these hypothermic levels the decreased metabolic activity constitutes or contributes to hypoxia of the cerebral tissues.

The reactivity of the cerebral vasculature to alternations in arterial $p\text{CO}_2$ is well known. It seemed logical, then, to see if this mechanism of reaction is functioning at hypothermic levels. If blood flow would be increased by raising the $\text{A}p\text{CO}_2$ in hypothermia, then a natural method for increasing flow to the cerebral tissues exists and more oxygen would be presented to the brain and could be extracted if hypoxia really existed.

The nitrous oxide method of Kety and Schmidt, slightly modified for hypothermic animals was used. Dogs were used in the majority of these studies; however, confirmatory results were obtained in five monkeys. The $\text{A}p\text{CO}_2$ was varied by inhaling various mixtures of CO_2 (2% and 5%).

In figure 1 the arterial $p\text{CO}_2$ is plotted on the abscissa and the cerebral blood flow on the ordinate. It is obvious that even at hypothermic levels the cerebral flow increases with increasing $\text{A}p\text{CO}_2$ values.

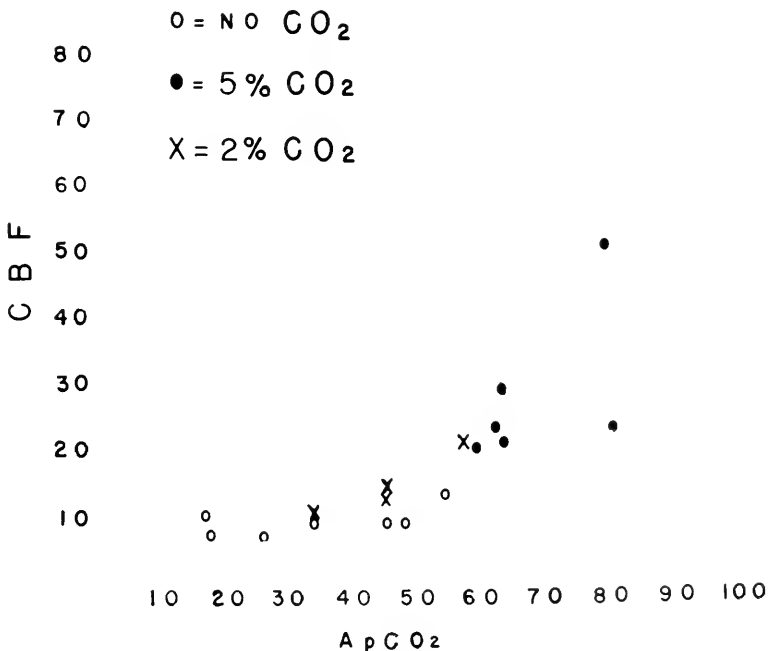


Fig. 1.—Effects of $\text{A}p\text{CO}_2$ on cerebral blood flow in hypothermia.

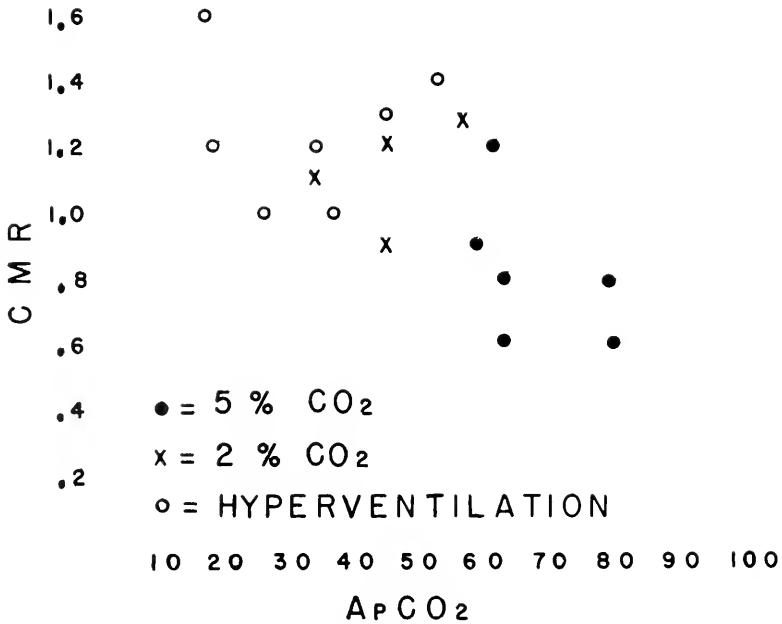


FIG. 2.—Effect of ApCO₂ on the cerebral metabolic rate in hypothermia.

In figure 2 the ApCO₂ is plotted on the abscissa and the cerebral metabolic rate (cerebral oxygen consumption) is plotted on the ordinate. It is clear that the animals with the highest ApCO₂ appear to have the lowest cerebral oxygen consumption. The reason for this is not entirely clear, but it may be that at the lowered levels of metabolic activity in hypothermia this concentration of CO₂ acts as an accessory anesthetic agent.

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HYPOTHERMIA AND THE CENTRAL NERVOUS SYSTEM

H. L. ROSOMOFF

You are probably familiar with the work of Dr. D. A. Holaday's and mine, published about a year ago, in which it was demonstrated that both cerebral blood flow and oxygen consumption decrease at the same rate during hypothermia in this temperature range. I present this as evidence to refute the statement of Dr. D'Amato in which he proposed that the peripheral circulation was a direct reflection of the cardiac output. The data in figure 1 would suggest that this is not true; it suggests that there is a mechanism of circulatory control within the brain.

This becomes more certain when the data in figure 2 are considered. The average cerebral blood flow falls at a rate of 6.7 per cent per degree Centigrade. The mean blood pressure decreases at a somewhat slower rate, 4.8 per cent per degree Centigrade. Therefore, cerebral vascular resistance must increase, and it does two- to three-fold.

The cerebral blood flow preparation is a method by which the blood flow is observed continuously so that one is able to follow constantly the dynamics of the cerebral circulation. It was interesting to note that for each temperature decrement

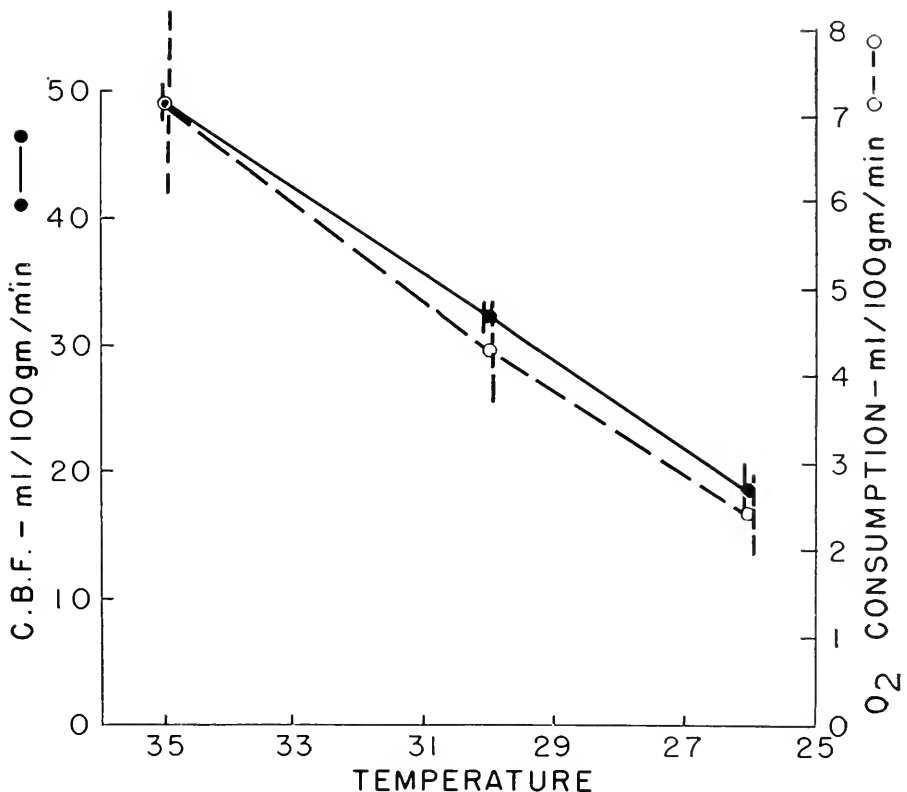


FIG. 1.—Decrease of cerebral blood flow and oxygen consumption with lowered temperature.

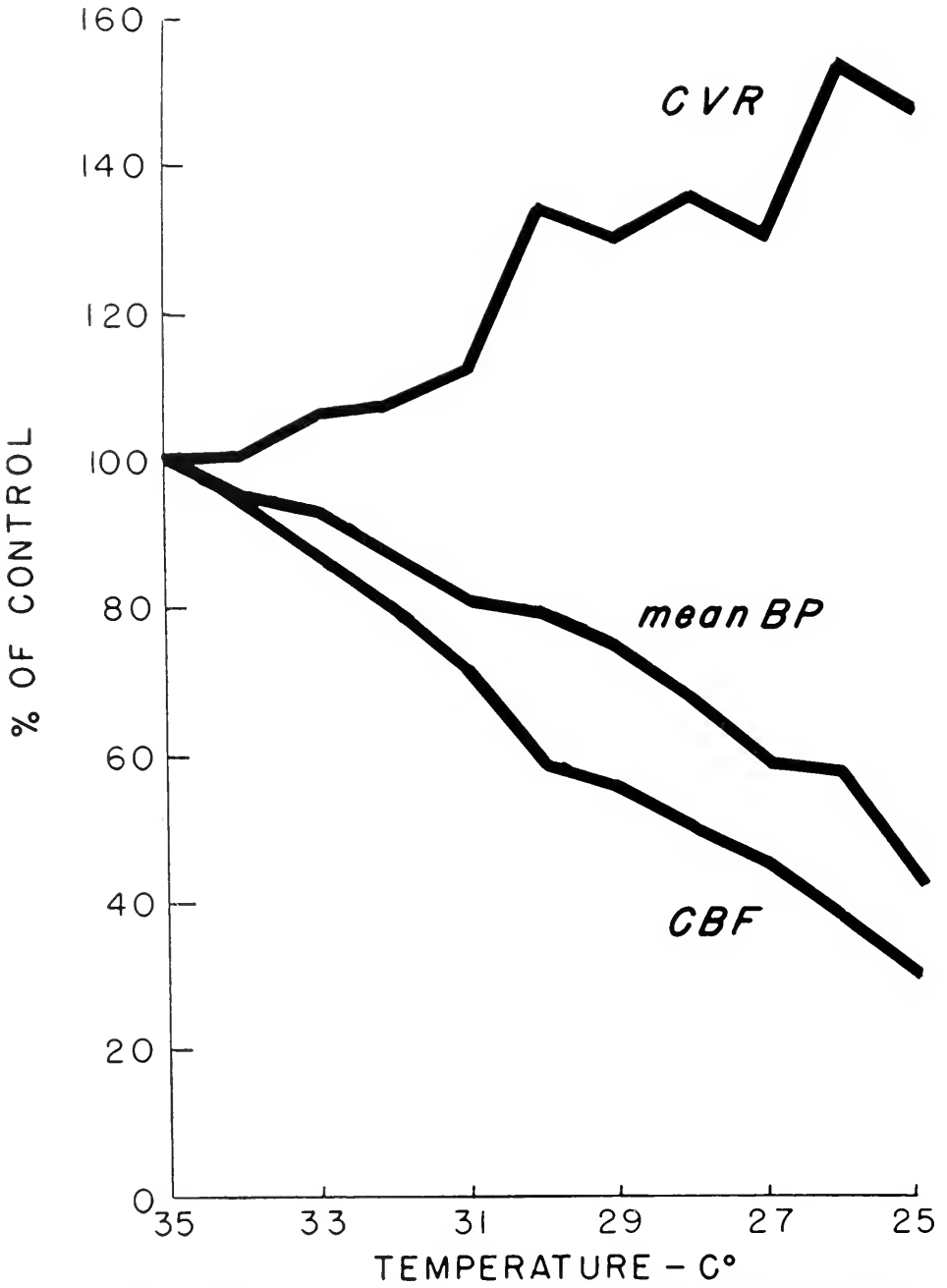


FIG. 2.—Changes in cerebral vascular resistance, mean blood pressure, and cerebral blood flow, measured in percentages of control values.

the cerebral blood flow fell at a constant rate, whereas the mean blood pressure fluctuated through wide extremes. Therefore, the cerebral vascular resistance was actively and rapidly varying in maintaining a constant cerebral blood flow in the face of a fluctuating blood pressure. This led to the conclusion that there must be a mechanism, mediated through the brain, by which this occurred and is most likely some function of the respiratory gas tensions in the blood.

It was noticed during the first set of experiments that the brain appeared smaller at 25° C. than at 37° C. We attempted to confirm this observation and to quantify the degree of change. Using a modification of the method of White for determining brain volume, the following results were obtained (table I). Attention is called to the last two columns. At 25° C. there is a decrease in brain volume of 4.1

TABLE I
DIFFERENTIAL INDICES OF BRAIN VOLUME, CHANGES IN EXTRACEREBRAL SPACE, AND
CHANGES IN BRAIN VOLUME OF NINE HYPOTHERMIC DOGS (25° C.)

Dog no.	Differential index of brain volume %	Change in extracerebral space %	Change in brain volume %
102	18.6	+68.9	-8.5
104	13.9	+26.3	-3.3
106	14.6	+32.2	-4.0
108	13.6	+23.8	-2.9
112	15.0	+36.2	-4.8
113	14.2	+28.6	-3.5
115	15.7	+42.4	-5.3
116	14.0	+27.0	-3.3
117	12.1	+ 9.9	-1.3
Means	14.4	+31.8	-4.1

per cent; whereas the extracerebral space, meaning that intracranial space not occupied by the brain, increases 31.8 per cent. The latter figure has a very important clinical significance since it represents the amount of space available for expansion of intracranial mass lesions.

Figure 3 shows an example of the cerebrospinal fluid pressure curves of nine animals during hypothermia. Note the elevation of pressure in one-half of the animals. This always occurred in the presence of shivering. When shivering was eliminated or was not present, there was a decline in the cerebrospinal fluid pressure.

Venous pressure and cerebrospinal fluid pressure were measured simultaneously (fig. 4). Both functions decreased at approximately the same rate, 5.5 per cent per degree Centigrade in this temperature range.

This has been a rather quick review of some of the effects of hypothermia on the normal physiology of the nervous system. Now to review the abnormal.

It has been well established that occlusion of the middle cerebral artery in the dog produces an infarct of significant magnitude. If the metabolism could be sufficiently reduced at the time of occlusion long enough for collateral circulation to establish itself, infarction might be minimized or prevented. It was proposed that hypothermia could achieve these conditions. Two series of experiments were con-

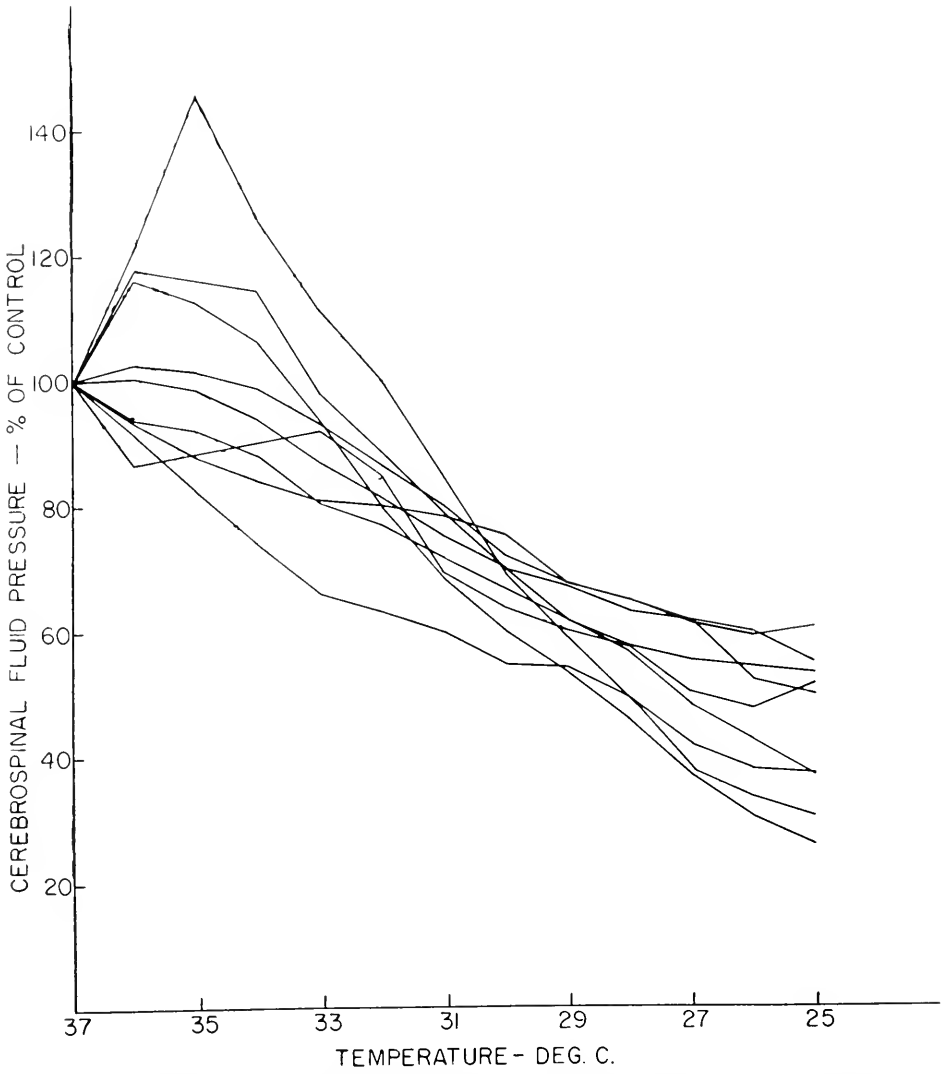


FIG. 3.—Cerebrospinal fluid pressure curves of nine animals subjected to hypothermia.

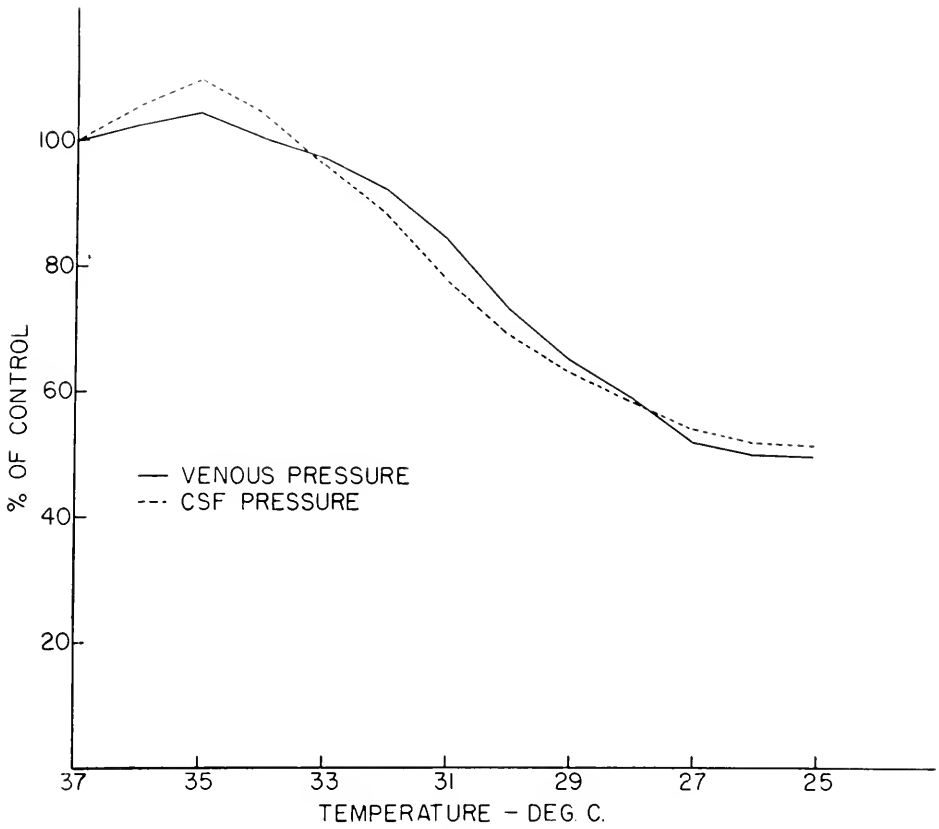


FIG. 4.

ducted; one in which the middle cerebral artery of the dog was transected at its origin at normal body temperature, and a second in which the same procedure was done during hypothermia of 22 to 24° C. Following division of the artery, the animal was rewarmed to normal body temperature, observed for three weeks, and then sacrificed.

Figure 5 shows the coronal sections of the brain of a normothermic dog following occlusion of the left middle cerebral artery. Note the severe degree of infarction.

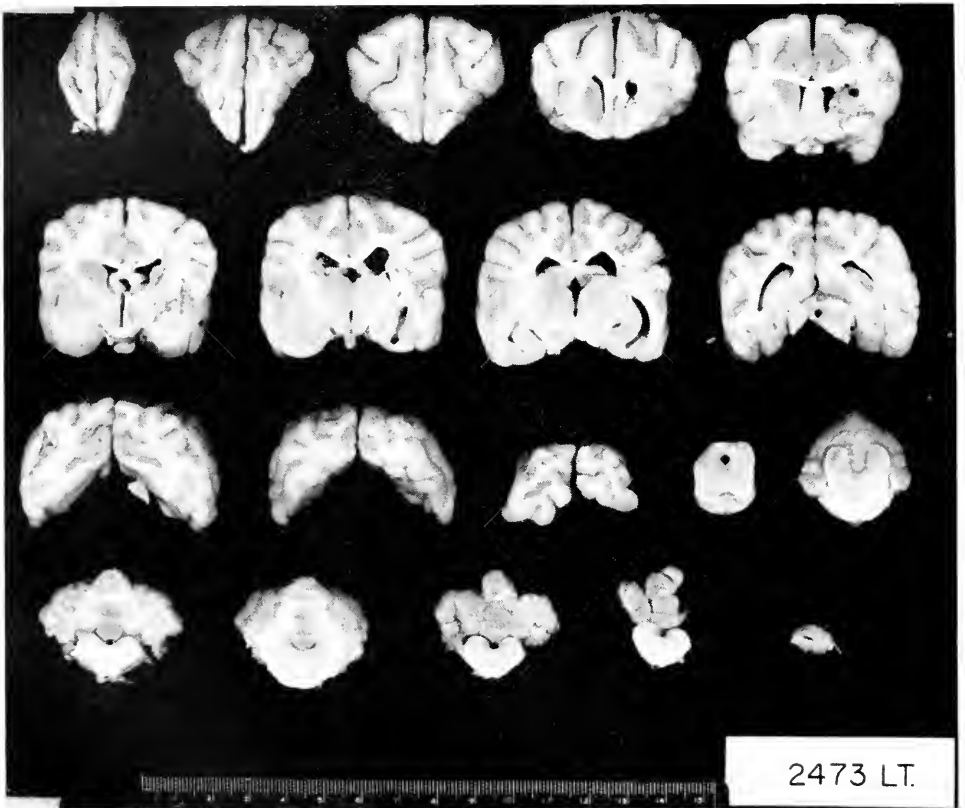


FIG. 5.—Coronal brain sections of normothermic dog with occluded cerebral artery.

Figure 6 shows the coronal section of a dog in which the left middle cerebral artery had been divided at 24° C. This brain is intact except for a 1×2 mm. area of infarction in the anterior hypothalamus which was not detectable clinically, and a traumatic subcortical cyst in the pyriform lobe which corresponds to the site of retraction at the time of operation. The remainder of the histology is normal. Therefore, we conclude that hypothermia can protect against cerebral infarction in the dog following occlusion of the middle cerebral artery.

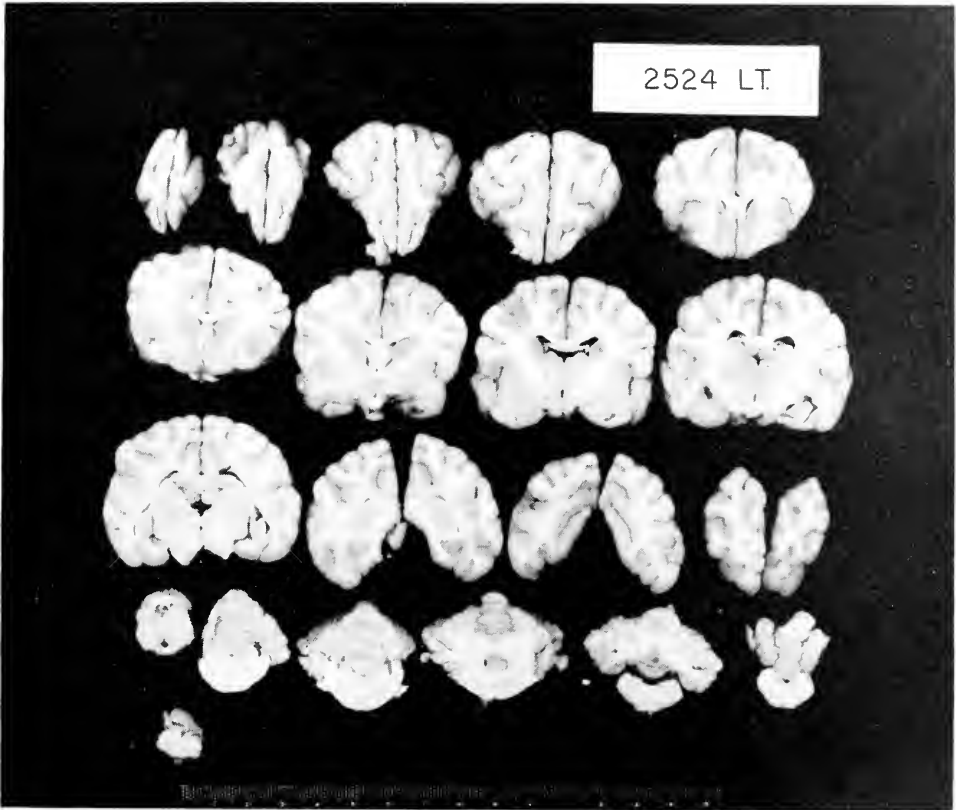


FIG. 6.—Coronal brain sections of hypothermic dog with occluded cerebral artery.

HYPOTHERMIA AND THE NERVOUS SYSTEM

CHANDLER McC. BROOKS

In discussion of Dr. McQueen's paper, I wish to amplify one or two points he touched upon and call attention to a series of phenomena which should be considered by those interested in hypothermia and its effects on the nervous system. I will mention these phenomena and make reference to the literature pertinent thereto.

The different susceptibilities of nerve fibers to cold block was mentioned. Large diametered A fibers block more readily than do B and C fibers and this is of importance in view of the functional roles played by fibers of these different categories. An additional point not mentioned is that afferent fibers as they fan out in the dorsal root are more readily blocked than they are in peripheral nerves. The nerve sheath and the blood supply are such that block of nerves by thermodes or general cooling is harder to achieve than is block of the dorsal (posterior) roots (Brooks and Koizumi, 1956).

A second point worthy of amplification is this: Cooling below normal body temperatures does decrease the excitability of nerves. Thresholds to applied stimuli are raised. However, when nerves cooled to a moderate degree ($38^{\circ} \rightarrow 25^{\circ}$ C.) are stimulated the action potentials evoked are enormously increased in amplitude and duration. The action potentials coming in over cooled afferent fibers have a much more potent central action. Cooling initially causes hypoexcitability but also *hyper-reactivity* of the nervous system. The cord must be cooled below 20° C. to reduce its responsiveness below normal levels (Brooks, Koizumi and Malcolm, 1955).

Cooling slows speed of conduction in peripheral nerve and spinal pathways. This is due to slow speed of rise of the action potential. The same occurrence explains slowed conduction in the hypothermic heart (see papers by Brooks and Hoffman in this volume). There is also selective block of conduction in the cord and a disproportionate effect on certain pathways (Brooks, Koizumi and Malcolm, 1955). Again fiber size and location provide at least partial explanation of this selectivity of action.

Not only does the higher amplitude and longer duration of the single action potential of a single afferent fiber constitute a greater central stimulus, but the train of impulses coming in over the afferent trunks has a longer duration. Due to the greater depressant action of cold on some fibers conduction is slowed more in these than in others; thus, afferent volleys are less well synchronized and act more like a tetanus. This gives one explanation of the greater responsiveness of the central nervous system in the cold (Koizumi, Malcolm and Brooks, 1954).

When one considers that the cooled interneurons are also acting in the same way one can understand another observation and that is that the motoneuron or reflex spike and discharge is increased much more in hypothermia than is the afferent potential (fig. 1, Brooks, Koizumi and Malcolm, 1955). This provides additional explanation of hyperresponsiveness.

Another interesting point is that the "purity" of reflexes tends to be lost in hypo-

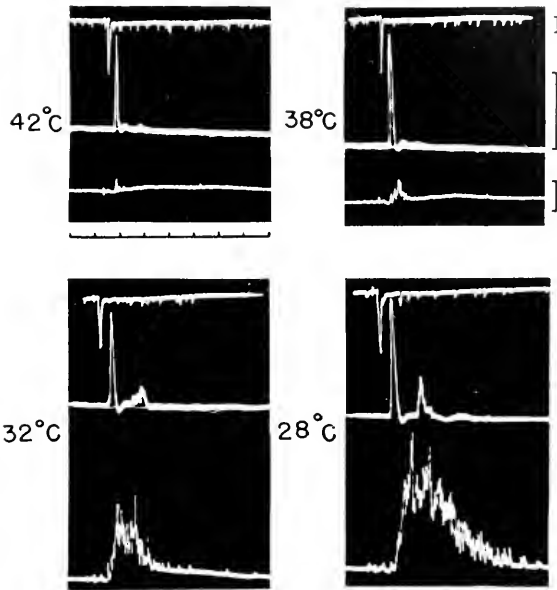


FIG. 1.—The effect of cooling the spinal cord on reflex response: Incoming volley (top beam), ventral root potentials (middle) and dorsal root reflex (lower). Time in 5 msec. intervals. Amplification signs = 1 mv.

thermia. A stimulus, such as a muscle stretch, which evokes a pure monosynaptic response at normal temperature elicits a reflex with a polysynaptic component if the spinal cord or the whole animal is cooled. Incoming impulses create central effects which “spill over” or involve other pathways not normally activated (fig. 2). In this connection it should be pointed out that polysynaptic reflexes are more greatly augmented by cooling than are the monosynaptic reflexes (Koizumi, Malcolm and Brooks, 1954).

This interaction during cooling plus the slowed conduction and greater amplitude of potentials in afferent terminals is probably responsible for the very great increase in dorsal root potentials and the dorsal root reflex (Brooks and Koizumi, 1955). The antidromic firing in adjacent roots and fibers occasioned by incoming volleys in the hypothermic animal is considerable and is capable of blocking by collision incoming afferent discharges. This may be of physiological importance to somatic response and might even contribute to phenomena such as ataxia and shivering.

As progressive cooling continues a stage is reached at which a single afferent volley evokes multiple motoneuron discharges. This repetitive firing of the cooled motor nerves is explainable on the basis of the prolonged and greater central action of the single stimulus. This is another example of hyperresponsiveness which is present though generally unseen because of the common use of anesthetics, the cooling of effectors and their reduced ability to respond.

Finally, it has been observed that cooling will produce a tetanus. The rhythm of this tetanic activity is much slower than that of a strychnine or metrazol-induced

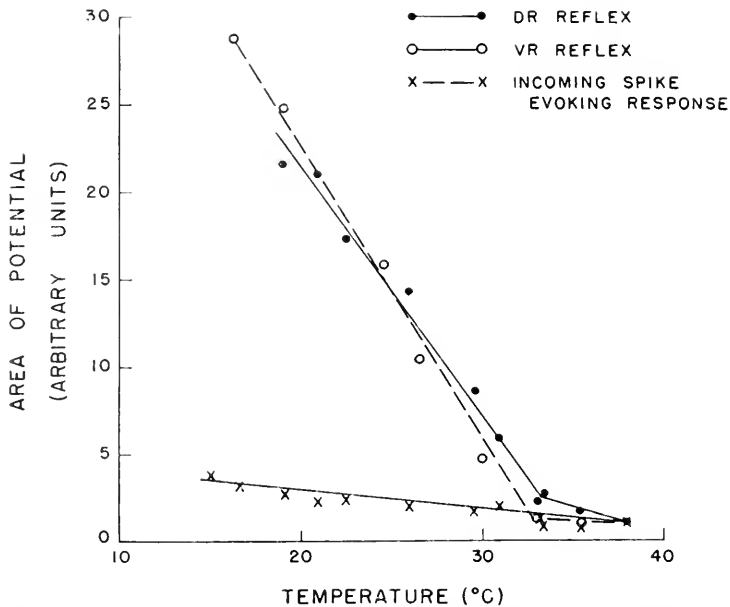


Fig. 2.—The effect of cooling the spinal cord on reflex response: Changes in magnitudes of responses in terms of amplitude and duration of potentials.

tetanus. Afferent stimulation will touch off the rhythmic firing but it will appear spontaneously even in a de-afferented cord (Koizumi, 1955). This observation of repetitive firing and actual tetanus in hypothermia is in line with the work mentioned by Dr. McQueen to the effect that hypothermia may aggravate epilepsy and certain types of neural seizures.

In conclusion I would like to reinforce my suggestion that the phase of hyperactivity which occurs before uniform depression sets in should not be left out of our considerations. Hyperactivity of centers may explain some peripheral phenomena. The relative timing of these phases in various brain and cord centers as an animal is cooled is not yet known. The origin of reported autonomic activity, metabolic changes, neuroendocrine discharges, the awakening from hibernation, etc. should be considered with hyperresponsive spill-over, as well as specific response pathways, in mind. The origin of shivering is not understood but explanation might come from a study of the effects of cold on the higher centers of the nervous system and hyperresponsiveness may be involved. As in the heart, temperature gradients are important because if gradients are sufficiently great an electrotonic current flow is established which will actually excite (Granit, 1955). Matters such as this have been discussed extensively in the neurophysiological literature but they are also relevant to behavior of heart cells and the origin of fibrillation.

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EXPERIMENTAL AND CLINICAL OBSERVATIONS ON THE USE OF HYPOTHERMIA TO PREVENT ISCHEMIC DAMAGE TO THE CENTRAL NERVOUS SYSTEM *

ROBERT G. PONTIUS AND MICHAEL E. DEBAKEY

The tissues of the central nervous system are particularly vulnerable to ischemic damage from even brief periods of circulatory arrest. This constitutes a serious hazard in the performance of certain cardiovascular procedures such as resection of aortic aneurysm, in which it may be necessary to interrupt aortic circulation for periods up to one hour.¹ Since it has been well established that hypothermia reduces total body metabolism and oxygen requirements of tissues, it is reasonable to assume that the central nervous system is similarly affected and that this might provide a useful measure in minimizing the ischemic dangers associated with these operative procedures.^{2,3} Accordingly, studies have been directed toward determining the protective value of hypothermia against such ischemic damage to the central nervous system during periods of temporary aortic occlusion.

Experiments along these lines have been done by a number of investigators as well as by us.^{1, 7-12} Thus, in a control group of 50 dogs occlusion of the aorta just distal to the left subclavian artery for a period of one hour was associated with an immediate mortality of 32 per cent and a paraplegia rate in the surviving animals of 65 per cent. The same procedure in a comparable group of 47 hypothermic dogs (body temperature was reduced to between 75 and 80° F.) was associated with an immediate mortality of 25 per cent and with a paraplegia rate of zero in the surviving animals (fig. 1). On the basis of these experiments as well as similar observations reported by other workers, the conclusion has been drawn that hypothermia has a protective influence against ischemic damage to the spinal cord following high aortic occlusion.

While the exact mechanism of protection afforded by hypothermia is not entirely clear, gross and histologic studies of the damaged spinal cord provide some clues to the problem. Gross examination of the spinal cords of the paraplegic animals showed bilateral symmetrical malacia of the grey matter of the lumbar, sacral, and coccygeal segments. Serial sections showed the lesions to begin consistently between T₁₂ and L₂ and continue distally. On microscopic examination pronounced changes were present in the grey matter, but the white matter was spared. As the specimens were obtained 12 days after injury evidence of repair by microglial phagocytes or gitter cells with foamy cytoplasm and proliferation of new capillaries through the destroyed area was present (figs. 2, 3, and 4). These changes are quite similar to those observed in the brain following anoxic damage to this organ. They do not suggest thrombosis or infarction as the vessel lumens were patent. The animals protected by hypothermia failed to show these changes.

Experiments along similar lines directed toward determining the protective effects of hypothermia against ischemic damage to the brain proved much more

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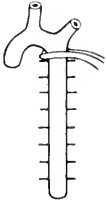
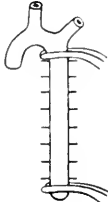
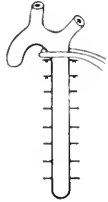
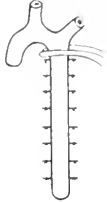
				
CONTROLS	Group 1	Group 2	Group 3	Group 4
Total Number	11	18	10	11
Died	1	8	2	5
Paraplegic	3	9	5	5
% Paraplegic	30% (3/10)	90% (9/10)	63% (5/8)	83% (5/6)
HYPOTHERMIC				
Total Number	10	18	10	9
Died	0	8	1	3
Paraplegic	0	0	0	0
% Paraplegic	0% (0/10)	0% (0/10)	0% (0/9)	0% (0/6)

FIG. 1.—Graph showing mortality for occlusion of descending thoracic aorta for one hour.

difficult, owing to the fact that in the dog extensive collateral circulation exists in the head and neck. Thus, occlusion of both carotid and vertebral arteries bilaterally produced no apparent disturbances. A preparation was finally developed, however, that resulted in a significant incidence of brain damage. This consisted in placing a silver clip on the basilar artery through the foramen magnum, applying occluding clamps to both carotid and vertebral arteries bilaterally and placing a tourniquet around the neck to produce compression of the muscular collateral vessels. In the control group of 9 dogs in which this was done for a period of 30 minutes, 6 (67 per cent) showed serious brain damage as manifested by convulsions, coma, and death. Significantly, none of the 9 hypothermic dogs treated in like fashion developed any neurologic disturbances. These observations, therefore, suggest that hypothermia is equally effective in preventing ischemic damage to the brain as to the spinal cord following temporary arrest of the circulation to these highly vulnerable tissues.

These experimental observations on the protective value of hypothermia in preventing ischemic damage to the central nervous system are supported by our clinical experience with its use in the excisional therapy of aortic aneurysm. In such cases in which the lesion is located in the thoracic aorta above the level of the seventh dorsal vertebra the procedure of resection and graft replacement is associated with the jeopardous effects of ischemic injury to the tissues of the

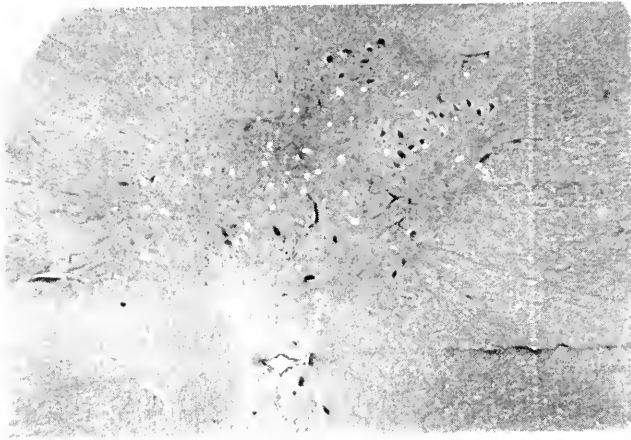


FIG. 2.—Microscopic section of normal spinal cord, L₂ level, of dog.

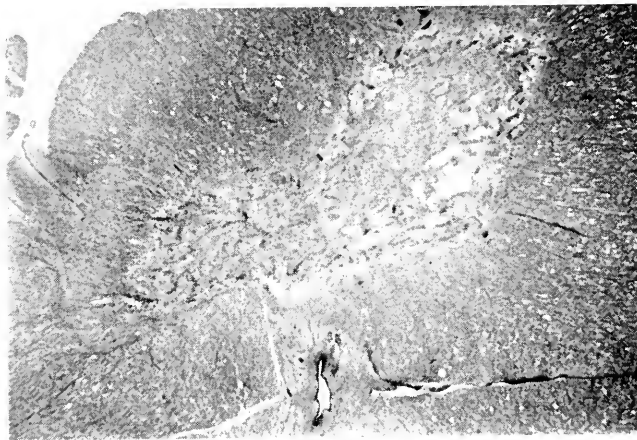


FIG. 3.—Microscopic section of spinal cord following aortic occlusion *without* hypothermia.

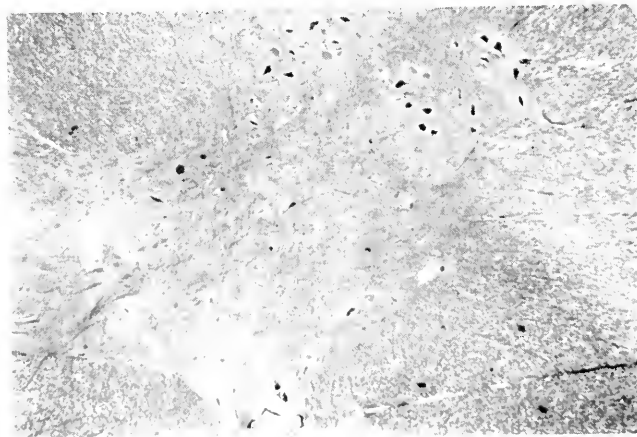


FIG. 4. Microscopic section of spinal cord following aortic occlusion *with* hypothermia.

central nervous system during the period of temporary interruption of aortic circulation. Thus, among the five cases of aneurysm of the aorta in our series, located at this high level and treated by resection without hypothermia, spinal cord damage occurred in four patients (fig. 5). Fortunately these changes were mild and transient in three, but probably contributed to the death of the fourth patient.

On the other hand, none of the 14 cases with comparable lesions similarly treated but in which hypothermia was employed showed any manifestations of spinal cord damage. Both in this as well as the former group the period of aortic occlusion averaged about one hour. In the hypothermic group body temperature was reduced to about 85° F. (figs. 6, 7, and 8).

Summary. 1. In a control group of 50 dogs in which the thoracic aorta was occluded just distal to the left subclavian artery for a period of one hour the immediate mortality was 32 per cent and the incidence of ischemic damage to the spinal cord as manifested by paraplegia in the surviving animals was 65 per cent. In a comparably treated group of 47 dogs in which hypothermia was used there was an immediate mortality of 25 per cent, but none developed paraplegia.

2. In a control series of 9 dogs in which the circulation to the brain was arrested for a period of 30 minutes evidence of ischemic damage to the brain occurred in 6 (67 per cent), but none of the 9 similarly treated group in which hypothermia was employed showed such manifestations.

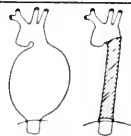
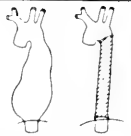
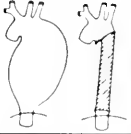

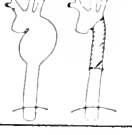
Case	Etiology	Date of Operation	Period of Occlusion	Location and Technic	Result
1. O.J. 50♂C.	Syphilis	6-11-55	48 min.		Transient paraplegia
2. T.M. 58♂W.	Syphilis	11-9-53	77 min.		Died-12 hrs. later Shock ?
3. M.W. 48♂C.	Syphilis	11-23-54	88 min.		Died-30 min. later Heart failure
4. M.F. 40♀W.	Dissection	1-11-55	34 min.		Transient paresthesias
5. M.H. 28♀W.	Traumatic	1-21-55	41 min.		Partial paraplegia

FIG. 5.—Chart of clinical cases *without* hypothermia.

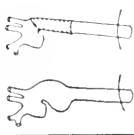
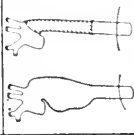
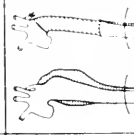
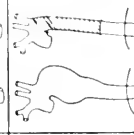
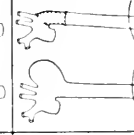
Case	Etiology	Date of Operation	Period of Occlusion	Location and Technic	Result
6. A.M. 63 ♀ C.	Syphilis	7-29-54	31 min.		Recovered
7. G.C. 43 ♂ W.	Syphilis	10-11-54	65 min.		Recovered
8. W.C. 46 ♂ W.	Dissection	11-23-54	65 min		Died-18 hrs later Ventricular fibrillation
9. C.B. 55 ♂ W.	Syphilis	4-8-55	35 min.		Recovered
10. M.B. 59 ♀ W.	Arterio- sclerotic	6-28-55	40 min.		Died-3 days later Cardiac failure

FIG. 7.—Chart of clinical cases *with* hypothermia.

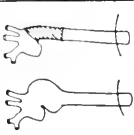
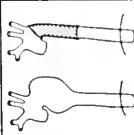
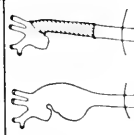
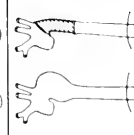
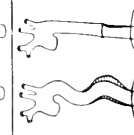
Case	Etiology	Date of Operation	Period of Occlusion	Location and Technic	Result
1. D.J. 18 ♀ W.	Congenital	1-1-54	53 min.		Died-1 week later Septicemia
2. M.M. 31 ♂ W.	Traumatic	2-5-54	62 min.		Recovered
3. J.B. 66 ♂ C.	Syphilis	3-4-54	54 min.		Died-8 hrs. later Secondary hemorrhage
4. F.V. 52 ♂ W.	Syphilis	7-5-54	58 min.		Recovered
5. P.M. 58 ♂ W.	Dissection	7-7-54	54 min.		Recovered

FIG. 6.—Chart of clinical cases *with* hypothermia.

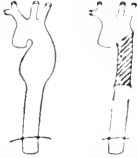
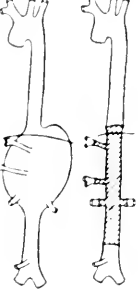
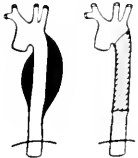
Case	Etiology	Date of Operation	Period of Occlusion	Location and Technic	Result
11 J S 53 ♂ W	Arterio-sclerotic	8-30-55	38 min		Recovered
12 J H 66 ♂ C	Syphilis	8-31-55	100 min		Died-1 week later Segment of infarcted bowel
13 W K 54 ♂ W	Dissection	9-15-55	53 min		Recovered

FIG. 8.—Chart of clinical cases with hypothermia.

3. Four of five patients with aneurysms of the thoracic aorta in whom the aorta was occluded for a period of about one hour developed evidence of spinal cord damage following resection. None of 14 similar cases in which hypothermia was employed developed any evidence of spinal cord damage.

4. On the basis of these experimental and clinical observations it would appear that hypothermia increases the tolerance of the tissues of the central nervous system to periods of temporary ischemia.

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DISCUSSION

Dr. W. H. Loughheed: I was interested in this paper because in our series of dogs, both the vertebral arteries and the common carotid arteries were occluded. If all the vessels arising from both subclavians were ligated as well as the internal mammary, then at normothermic temperatures these dogs showed cerebral damage. However, if they were hypothermic, reduced to 25 degrees Centigrade, we found they were protected from 15 to 17 minutes.

At 25° C. these animals had cerebral metabolic rates varying from 35 to 25% of normal. This would indicate that they should withstand a period of anoxia 3 to 4 times longer than at normal body temperature.

POSSIBILITIES AND LIMITATIONS OF DIFFERENTIAL BRAIN COOLING IN DOGS *

JAY M. JENSEN,† W. M. PARKINS AND H. M. VARS

It is the paradox of hypothermia that both the promise and the limitation of the method lie in the same direction. The central nervous system is protected by cold against ischemia and, within certain limits, the measure of protection seems to increase as the hypothermia deepens. The myocardium is endangered by cold and its vulnerability to arrest and to arrhythmia seems to become greater as the temperature falls. In the dog and in the human this riddle remains. If these phenomena could be studied separately perhaps additional data might be obtained about both. This was attempted by cooling the brain, the heart, and the body core somewhat selectively.

Procedure. The cooling system consisted of an ice bath, a polyvinyl coil, and a pump. Adult, mongrel dogs were anesthetized with sodium pentobarbital. An endotracheal tube was placed. The pump was adjusted to deliver through the coil approximately 100 ml. of blood per minute.

The carotid artery on one side was exposed and ligated. Polyvinyl catheters were placed above and below the ligatures with the proximal catheter directed toward the heart and the distal catheter directed toward the brain. The catheters were connected to the coil so that oxygenated blood would be pumped from the animal, through the polyvinyl coil immersed in ice, and pumped back into the carotid and through the brain. Small holes were drilled into the skull and through these copper-constantan thermocouples were inserted into the cortex of the frontal lobes. Base line recordings were taken, the perfusion pump was started, and differential cooling of the animal was begun.

Most of the animals were heparinized after completion of the operative procedures. In several instances, however, the heparin was inadvertently not given and only rarely was there evidence of clotting within the coil. The temperature of the blood entering the carotid artery from the iced coil ranged from 3° to 10° C. The cooling period extended from 10 to 20 minutes. In certain of the animals the chest was opened and the inflow tract was occluded. At the conclusion of the procedures the animals were warmed in a warm water bath.

The measurements shown on the accompanying graph were made during the differential cooling of a dog. The rectal temperature is shown on the top line. The brain temperatures were recorded on the perfused and on the unperfused sides. These factors were recorded during 15 minutes of cooling, during 30 minutes of complete occlusion of the general circulation, and during the immediate recovery period. This dog tolerated 30 minutes of complete occlusion of the superior and inferior vena cava without evidence of damage of any kind (fig. 1).

Results. In 15 dogs the general circulation was completely interrupted by clamping the inflow tracts. The interval of occlusion varied from 15 to 40 minutes.

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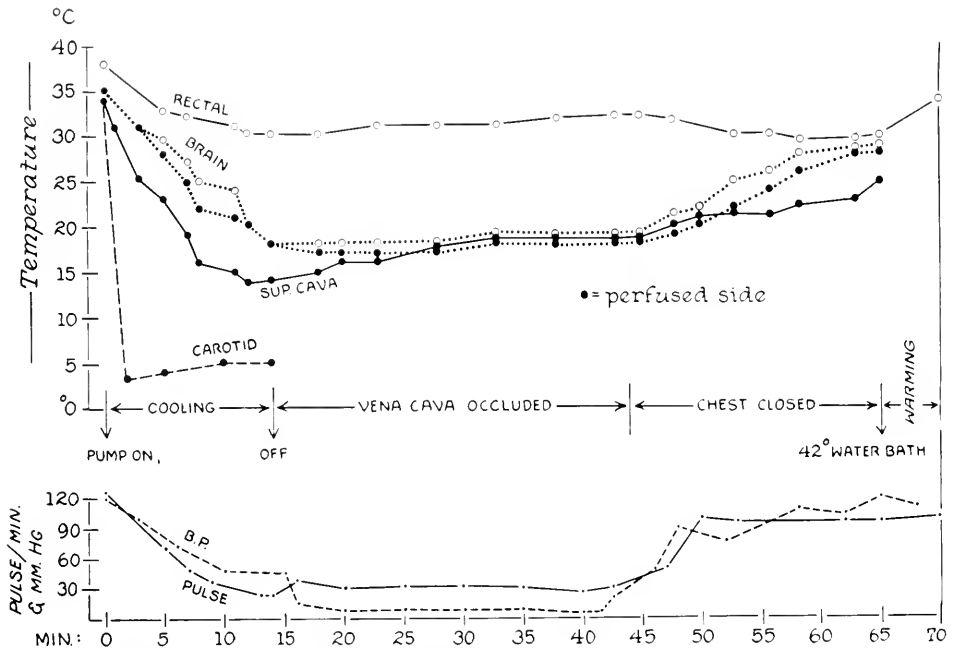


FIG. 1.—Pulse, blood pressure and differential temperatures of a dog during 15 minutes of perfusion cooling, 30 minutes of occlusion of the venae cavae and 25 minutes of the recovery period.

TABLE I

VENA CAVAL OCCLUSION IN DIFFERENTIALLY COOLED DOGS

Dog no.	Lowest brain temperature		Lowest rectal temp.	Time occluded	Result
	Perfused side	Unperfused side			
46.....	17° C.	23° C.	32° C.	15 min.	Living and well
48.....	—	—	32° C.	15 min.	Living and well
53.....	—	—	32° C.	15 min.	Living and well
24.....	18° C.	19° C.	30° C.	20 min.	Living and well
43.....	16° C.	21° C.	32° C.	25 min.	Living and well
52.....	18° C.	20° C.	32° C.	25 min.	Living and well
25.....	15° C.	20° C.	30° C.	30 min.	Living and well
26.....	18° C.	18° C.	29° C.	30 min.	Living and well
49.....	—	—	32° C.	30 min.	Living and well
50.....	—	—	34° C.	35 min.	Living and well
28.....	18° C.	20° C.	31° C.	40 min.	Died in 24 hrs.

Certain of the animals seemed to tolerate this ordeal better than others but in each case the heart continued to beat slowly and regularly during the interval of occlusion. Ten dogs shown in table I recovered without discernible damage. One dog, cooled to a rectal temperature of only 31° C. and in which the general circulation was interrupted for more than 40 minutes did not regain consciousness and died after 24 hours. This animal passed bloody mucus per rectum during the immediate post-operative period. At autopsy the bowel was hemorrhagic and contained bloody fluid.

Three animals, shown in table II, developed fatal cardiac arrhythmias as the occluded venae cavae were being released. Two developed rapid fibrillation imme-

TABLE II

VENA CAVAL OCCLUSION IN DIFFERENTIALLY COOLED DOGS: CARDIAC DEATHS

Dog no.	Lowest brain temperature		Lowest rectal temp.	Time occluded	Result
	Perfused side	Unperfused side			
12.....	15° C.	18° C.	30° C.	15 min.	Died. Fibrillation
27.....	18° C.	—	29° C.	20 min.	Died. Fibrillation
32.....	20° C.	20° C.	31° C.	25 min.	Died. Asystole

TABLE III

DIFFERENTIAL COOLING IN DOGS (NO OCCLUSION)

(Brain temperatures reduced to 12°–18° C. No neurological damage.)

Dog no.	Lowest brain temperature		Lowest rectal temp.	Result	
	Perfused side	Unperfused side		Alive	Defect
20.....	13° C.	14° C.	25° C.	Yes	No
22.....	12° C.	12° C.	27° C.	Yes	No
24.....	18° C.	19° C.	30° C.	Yes	No
25.....	15° C.	20° C.	30° C.	Yes	No
26.....	18° C.	18° C.	29° C.	Yes	No
33.....	14° C.	17° C.	24° C.	Yes	No
37.....	16° C.	21° C.	27° C.	Yes	No
39.....	18° C.	24° C.	26° C.	No	Arrest

TABLE IV

DIFFERENTIAL COOLING IN DOGS (NO OCCLUSION)

(Brain temperatures reduced to 8°–12° C. Neurological damage.)

Dog no.	Lowest brain temperature		Lowest rectal temp.	Result
	Perfused side	Unperfused side		
16.....	10° C.	12° C.	27° C.	Disoriented
17.....	9° C.	—	26° C.	Motor ataxia
18.....	9° C.	—	26° C.	Motor ataxia
23.....	12° C.	13° C.	25° C.	Motor ataxia
30.....	8° C.	10° C.	26° C.	Motor ataxia
16.....	10° C.	12° C.	27° C.	Disoriented
14.....	9° C.	10° C.	28° C.	Comatose. Died
15.....	8° C.	10° C.	24° C.	Comatose. Died

diately while one developed an asystole which reverted to fibrillation. Cardiac massage, electrical defibrillation, and appropriate drugs were tried but in these animals a normal, effective rhythm was not re-established. In addition two animals not shown recovered initially but died during the post-operative period of undetermined causes.

In 16 animals the circulation was not occluded. Eight of these, shown in table III, were subjected to brain temperatures of from 12° to 18° C. One animal died during the cooling period but the remaining seven recovered completely. Eight dogs, shown in table IV, were cooled differentially for long periods of time and to brain temperatures ranging from 8° to 12° C. There was evidence of gross sensory and motor disturbances in all of these. Two animals remained comatose and died after several days. In each case, gross examination of the brain was unrewarding. Four dogs

suffered motor ataxia and two were markedly confused. These motor and sensory difficulties seemed to rapidly improve over several days but residuum remained for several months.

Discussion. The possibility of protecting tissues by cold has initiated extensive laboratory and clinical investigation. Considerable protection can be afforded the central nervous system and the measure of this is being rapidly defined.^{1, 2, 3, 4, 5, 6} These experiments indicate that brain temperatures of 20° C. with general body temperatures of 30° C. will protect the dog against 30 minutes of complete occlusion of the circulation. Others have subjected dogs to general body temperatures lower than the brain temperatures recorded here without evidence of neurological damage.⁷ Under the conditions of these experiments, however, neurological damage was encountered at very low brain temperatures.

The dog can be cooled by the method described to a brain temperature of 20° C. and a rectal temperature of 32° C. within 15 to 20 minutes. It is believed that this method of cooling might better reveal the separate effects of cold upon the organism. It is expected that in future experiments the catheters can be manipulated into position without endangering the carotid artery.

The cold heart is vulnerable to arrhythmias and the experience of many in applying resuscitative measures has been disappointing.^{8, 9} This liability seems to be compounded by operative manipulation and especially at lower temperatures. In the selectively cooled animal with the heart relatively warmer we believe that these difficulties may be significantly less.

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DISCUSSION

Dr. W. M. Parkins: Dr. Vars, Dr. Ben, and I have been interested in the relative role of the liver and the intestine in the pathogenesis of ischemic shock induced by temporary occlusion of the thoracic aorta, and the influence of hypothermia in extending the tolerance time to such vascular occlusion.

Early exploratory experiments revealed that maximal incidence of paraplegia, shock, and death occurred when a balloon catheter occlusion was positioned at the level of the mid descending thoracic aorta, i.e., between the 8th and 10th intervertebral space. Eighty per cent of these dogs survived 30 minutes of occlusion; 50 per cent of which became paraplegic. Forty per cent survived 1 hour of occlusion, with all paralyzed in the hind quarters. Following 2 hours of occlusion, death occurred in all animals between 2 and 6 hours after release of the obstruction.

Surface cooling to a rectal temperature of 30° C. was found highly effective in protecting the spinal cord from anoxial damage during a 1-hour period of occlusion. None of these hypothermic animals were paralyzed. There was no increase, however, in survival rates compared to that of the controls.

A similar result was obtained by generalized hypothermia induced by blood refrigeration reducing the rectal temperature to 30° C. When the temperature was reduced to still lower levels (24–27°) by blood refrigeration, the first indication of protection against the hemoconcentration, hypotension, shock, and death was obtained. Seven of 11 dogs survived 1 hour of occlusion without paraplegia.

If liver ischemia were the primary factor limiting the time of tolerance to occlusion, liver arterialization by a carotid-portal shunt should be effective. By immersion of the shunt in ice water, the temperature of the liver was differentially reduced from the body mass to levels below those obtained with generalized hypothermia (liver 20°—rectal 30° C.). Liver arterialization alone did not ameliorate the shock-like state which followed a 1-hour period of occlusion; only two of nine survived. A moderate protection against shock was indicated in the hypothermic animals with five of eight surviving 72 hours with complete recovery. None of the hypothermic survivors was paraplegic.

In view of the negative result with liver arterialization, and noting the correlation of intestinal infarction, bloody diarrhea, and profuse sloughing of the mucosa with mortality, our attention was focused upon the intestine. Differential cooling of the intestine was accomplished by rapid filling of the abdominal cavity with iced saline. In initial experiments, saline was recirculated through a coil immersed in an ice water bath. This was done to maintain the temperature of the duodenum at 10–15° or 20°, while the liver temperature was about 25°, and organs central to occlusion about 30°.

The first four animals were occluded simultaneously with administration of the iced saline. After 1 hour of occlusion all survived 72 hours. Hemoconcentration and hypotension were minimal; bloody diarrhea and mucosal sloughing were not observed in these animals. Their recovery was prompt, without paraplegia, and with a wide margin of safety.

We proceeded, therefore, to a 2-hour interval of occlusion in 10 animals. Six animals were occluded at the beginning of cooling, five of which survived, one being paraplegic. In four animals the cooling was delayed until 15 minutes after the occlusion. All four survived; 2, however, were paralyzed in the hind quarters.

To simplify this method of direct visceral cooling, the iced saline (65 cc./kg.) was injected interperitoneally within two to three minutes. This cooling was supplemented by placing an ice pack on the lower abdomen. Five of 5 animals occluded for 1 hour simultaneously with cooling, survived; 2 being paraplegic. Five of 5 survived a 2-hour period of occlusion. Three of these, however, were paraplegic.

Ten dogs were precooled to a rectal temperature of about 25° C. by this method

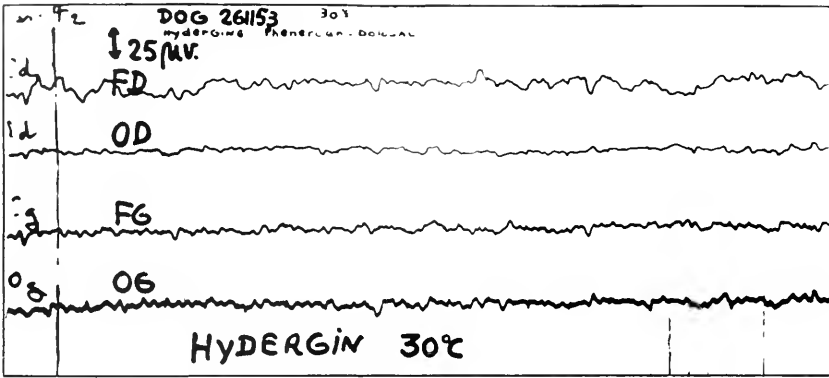


FIG. 1.

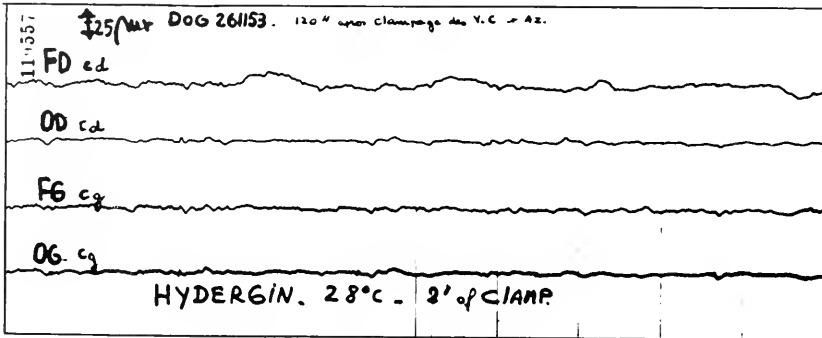


FIG. 2.

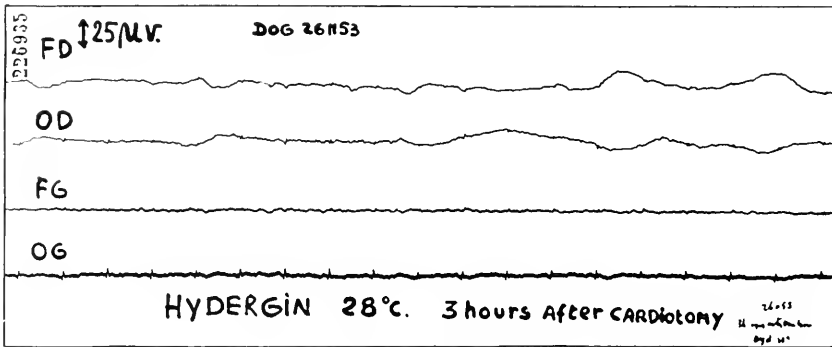


FIG. 3.

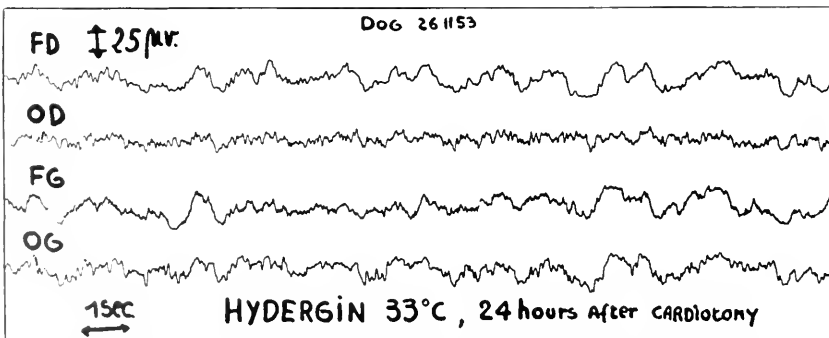


FIG. 4.

Figures of Dr. Jean Calin: EEG tracings during surgery on the bloodless heart in drug-induced hypothermia.

prior to occlusion. Seven of 10 survived a 2-hour period of occlusion. None of these were paraplegic.

As additional normothermic controls, 37-degree saline was substituted for the iced saline. Eight of 10 animals survived 1 hour of occlusion, thus suggesting a beneficial effect of the saline alone. All survivors were paraplegic. None of 10, however, survived the 2-hour occlusion.

The integrity of the intestine appears to be a primary factor in survival following temporary occlusion of the thoracic aorta. Direct visceral hypothermia was the most effective procedure in prevention of this type of ischemic shock.

Dr. Jean Cahn: Figure 1 (page 226) shows the encephalographic control during total arrest of the blood circulation. That is a usual encephalogram at 30° C., regardless of the method of hypothermia employed.

Silence of the brain is obtained 30 to 120 seconds after arrest of the blood circulation (fig. 2), depending on the method of the hypothermia. It seems that silence is obtained after only one or two minutes in hibernation, and is obtained in 30 to 60 seconds in surface cooling. After 20 or 25 minutes of circulatory arrest, when the clamp is released and the heart rate and the blood pressure returned to normal, silence in the brain persists for two to four hours after release of the clamp (fig. 3). Twenty-four hours later, when the temperature has returned to 33° C., the electroencephalogram is normal (fig. 4).

Silence is obtained after 120 seconds at 27° C., and after 80 seconds at 29° C. After clamping, the number of slow waves of small amplitude is increased. In only one case did we find fast waves. The return to the fast waves is about 120 seconds to 200 seconds after the release of the clamping. I can't understand why, despite the return to normal of the blood pressure, silence in the brain persists after 20 minutes of clamping.

Dr. James D. McMurrey: We have been interested in a study of cerebral physiology under hypothermia and in the possibility of occluding the cerebral afferent circulation in monkeys. We have occluded the cerebral afferent circulation under hypothermia in about 60 monkeys by an occlusion of the brachycephalic and left subclavian arteries at the arch of the aorta. Almost invariably if the occlusion were complete there was disappearance of the electroencephalographic tracing within one minute.

Figure 1 (page 278) is a reproduction of electroencephalographic tracings in a monkey subjected to 15 minutes of cerebral afferent vascular occlusion under hypothermia. We believe that occlusion of the brachycephalic and left subclavian vessels is sufficient to completely occlude the cerebral afferent circuit. Almost invariably we got the pattern demonstrated here, which is a marked depression to almost complete absence of electrical activity during cerebral afferent vascular occlusion.

In a series of monkeys with occlusion of the cerebral afferent circulation for more than 20 minutes, the animals either died or, upon follow-up by clinical examination and encephalography, were found to be greatly damaged.

Monkeys occluded for 15 minutes seem to tolerate the procedure moderately well. We were able to produce multiple occlusions for as many as three periods of occlusions, with five minutes' interruption of the occluding period, for periods of 12 minutes without damage.

Studies of the cerebral oxygen consumption and cerebral blood flow demon-

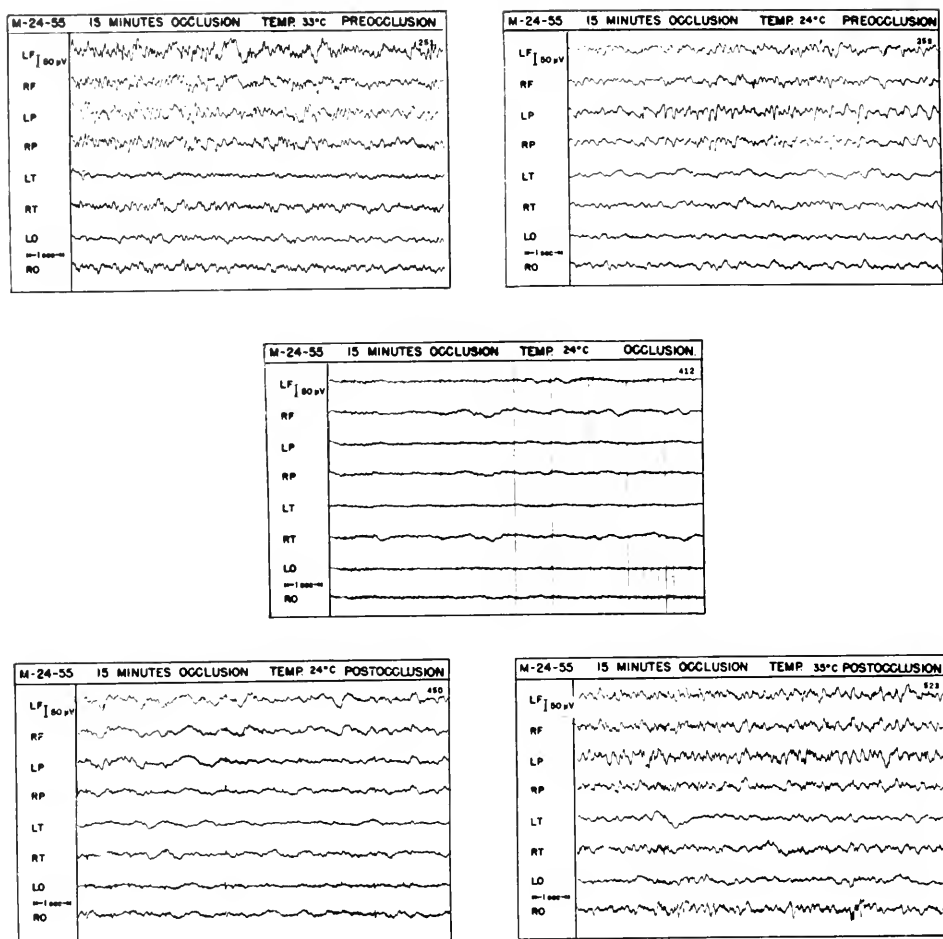


FIG. 1.—Photograph of a series of electroencephalographic tracings obtained in a monkey subjected to 15 minutes of cerebral afferent occlusion at temperature 24°C . The tracings read from left to right. The first tracing is a control at 33°C .; the second is at 24°C . before occlusion; the third is at 24°C . during occlusion (note the marked depression of electrical activity); the fourth is at 24°C . after release of occluding clamps; and the fifth is at 35°C . after rewarming. (Dr. James D. McMurrey)

strated a markedly diminished oxygen utilization, in the range of 31 to 27°C . without much change below that temperature range. We felt that it was unnecessary to take our animals below 27°C . in order to achieve a near minimal utilization of oxygen.

In some of our monkeys we observed that the electroencephalographic tracing did not completely disappear. Sometimes we were able to produce complete disappearance by readjustment of the clamps, and in one or two of our monkeys we discovered that there was an afferent vessel not occluded at the time of the original application of the clamps. We have concluded that the presence of a minimal amount of blood flow to the brain was sufficient to protect these animals from damage with marked reduction of cerebral blood flow at low body temperature.

REVIEW AND APPRAISAL OF PARTS I AND II

J. W. SEVERINGHAUS

This synopsis will attempt to present the more important concepts which may now be assumed to be substantiated in this field. Mention will be made of misleading or improbable statements contained within the manuscripts of the various participants, lest these otherwise be accepted as valid. Contradictory results will be contrasted and some areas needing further research will be noted. Obviously, much that is important and undisputed cannot be summarized here, since many of the participants have prepared comprehensive reviews and summaries of work in their particular field.

The fields of emphasis which emerged at the conference differed slightly from the organization of the agenda. Therefore, this discussion will be organized to highlight several subjects not specifically mentioned by title: such as Temperature gradients, Temperature regulation, Endocrine and Electrolytes.

Metabolism. It now seems generally agreed that body metabolism diminishes as a direct effect of low temperature, and that none of this fall represents oxygen or substrate deprivation in the cellular environment. For the whole body the extent of this decrease in metabolism at 30° C. appears to vary from 25–50%. It was suggested that cold may stimulate metabolism by some non-shivering mechanism in the dog. It seems possible that occult shivering and/or vasoconstriction have not been eliminated as contributing factors in the partially poikilothermic animal. The inference drawn by the reviewer is that the lowest metabolic rates will obtain when sympathetic nervous system activity and epinephrine release, as well as shivering, are blocked. In general, the metabolic activity of each organ studied was found to diminish at about the same rate. However, the effect of this on the organ function varies. For example, liver oxygen consumption at 24° was reported as 40% of normal, but the half life of morphine was increased from 3.7 to 94 minutes. Conversely, water and sodium excretion by the kidney are not depressed at low temperatures, since their reabsorption by the distal tubule appears to be depressed first. Furthermore, nerve action potentials are actually increased at low temperatures, and certain aspects of the central nervous system may be hyper-reactive. The heart was reported to exhibit a greater efficiency and a near normal stroke volume at 28° C. Thus, it cannot be said that all body functions are uniformly depressed by low temperatures. Whereas a few reports suggest that metabolism falls linearly with temperature, leading to the unlikely conclusion that metabolism is zero at 10°, most reports suggest the expected exponential fall off, the hibernators retaining 4–10% of their normal rate at 4° C.

A comment on the frequent observation of RQ values below 0.7 during hypothermia is needed. The marked changes in solubility of CO₂, respiration, pH and blood bicarbonate levels have often not been adequately considered. It must be recognized that CO₂ is gradually stored in the body instead of being eliminated. It seems doubtful that any true tissue RQ falls below .70, although the respiratory exchange ratio, R, may be very low for long periods of time.

Temperature gradients. Although little mention was made of the existence of temperature gradients in the deep parts of the body during hypothermia, this field needs more careful study. The rectal and colonic temperature, although the standard reference for most work, have been found by us to be as much as 12° C. lower than the central aortic and esophageal temperatures during rapid surface cooling after vagotomy, and 4° C. higher or lower than esophageal temperatures in man during surface cooling. If hypothermia is to be a study of function versus temperature it is manifest that a correct estimation of central body temperature is needed. The reviewer would conclude that best access to this temperature is in the lower esophagus adjacent to the heart. In our experience the esophageal temperature was found to correlate much better than rectal temperature with oxygen consumption.

Temperature regulation. The temperature regulation center appears to have been accurately localized in dogs to the posterior hypothalamic gray matter. When this area or its connection to the mid brain was destroyed, poikilothermic animals were obtained who showed normal basal metabolic rates at 37°. It was noted that destruction of either of two endocrine centers, the anterior hypothalamus and the pituitary, lowers the basal metabolic rate, but transection of the pituitary stalk joining them does not. Recent evidence suggests that the dog retains some temperature regulation even at 24° C. during prolonged hypothermia.

Nervous system. A considerable part of the interest in hypothermia is in protecting the central nervous system from ischemic damage, yet relatively little reported work defines the exact parameters of safe time, or of damage at various intervals of time. If one may assume that the higher centers are most easily damaged, it would seem that post-hypothermia cerebral damage should not be judged by ataxia or coma, but by the finer psychometric determinations available today both for animals and man. Psychologists and psychiatrists should find interesting material in post-hypothermic patients which to date have largely been judged by relatively crude tests.

There is considerable disagreement about the damage to nervous tissue that may be produced by low temperatures. One group pumped cold blood into one carotid artery in the dog and found damage when the brain temperature fell below 12° C. on the colder side. However, the pressure used to perfuse was not reported, and either excess pressure, the extremely low pCO₂ in the perfusate, the high pH, currents resulting from local extreme cold, or temperature gradients, might be implicated. Other workers have cooled monkeys and dogs to lower temperatures without observing post-hypothermia brain damage. Peripheral nerve palsies can occur from prolonged ice water immersion, without frostbite. The recovery of electrical activity of the cortex after 1 minute of ischemia is reported to be most rapid at about 30° C.; however, to assume that this is therefore the safest temperature for clinical hypothermia with blood flow occlusion is unjustified, since the slower recovery at lower temperatures may not indicate increased damage.

A new approach to the analysis of brain activity with low temperatures was described in connection with awake poikilothermic dogs. These unanesthetized animals could be observed during cooling, and were found to function surprisingly well, showing emotional and physical responses only slightly depressed, and eating

food at rectal temperatures of 28° C. A comment on this work suggested that rectal temperature might be seriously in error in these dogs with neurologic lesions since cervical vagotomy had been shown to cause marked differential cooling of the rectum.

The EEG is said to exhibit large delta waves at about 30° C., falling to electrical silence at 18° C. Recovery of electrical activity may require several hours after 20 minutes of ischemia at 28° C. (and about 1 hour after 6-8 minutes at 28° C. in the reviewer's experience). Cerebral seizures reported during hypothermia are believed explained by brain hyper-reactivity and the larger and more spread-out afferent volleys from the periphery.

Cerebral hemodynamics have received considerable attention. Several workers report increased cerebral blood flow in response to elevated pCO₂ in dogs at 25-28°. A suggestion that elevated pCO₂ decreased cerebral oxygen consumption seems unwarranted in view of the scatter of experimental data and the possible fall in brain temperature with increased perfusion rates during cooling. Even at low temperatures cerebral blood flow regulates according to the brain's metabolic needs.

Hemodynamics. A curious observation is that, whereas pulse rate declines linearly with fall in temperature, arterial pressure falls only gradually to about 24° C., and then considerably more rapidly at lower temperatures. The bradycardia at low temperatures is not like vagal bradycardia, and is not altered (at 25° C.) by atropine or vagotomy. Stroke volume is nearly normal down to 20-25° C., and the ventricular filling pressure is not elevated. Coronary flow has been shown adequate for the needs of the heart by a number of investigators, although the coronary vascular resistance is highly dependent on arterial pressure at low temperatures (reduced resistance resulting from high pressures). Although peripheral capillary stasis has been noted, no evident oxygen debt occurs, so the consensus is that peripheral circulation is also adequate while the heart continues to beat.

Post-hypothermia circulatory insufficiency was noted by several authors and remains an unsolved problem. Factors which seem most prominent as possible causes are two: the peripheral vasodilatation during rewarming, with resultant increased tissue oxygen utilization, and possible adrenal insufficiency resulting from the lack of adrenal response to trauma during hypothermia, perhaps augmented by renal sodium loss.

Hematology. A marked thrombocytopenia has been observed by many investigators in dogs, and by one in monkeys. Reports in man were limited to two patients who showed small decreases at moderate temperatures. This thrombocytopenia, it was suggested, could be prevented by the ganglionic blocking drug, Arfonad, or by blood stream cooling instead of surface cooling in dogs. The mechanism of these interesting observations was not discussed. The platelets were shown to sequester in the liver and spleen, and possibly the gut, and to reappear on rewarming.

Respiration. The errors and difficulties in correcting blood gas tensions for the physico-chemical effects of low temperatures have been responsible for a number of erroneous reports in this field. For example, the oxygen tension of blood in a syringe falls 6% per degree temperature fall; pK' was found not to be constant,

but to vary with pH as well as with temperature. Although the oxygen dissociation curve shifts to the left at low temperatures, this has not been shown to result in tissue anoxia. The now common use of hyperventilation raises the pH and further decreases the dissociation of O_2 from hemoglobin and more careful observation of the possibility of cerebral and myocardial ischemic effects is needed. Particularly in the brain this may be of importance since the concomitant decrease in arterial pCO_2 will diminish cerebral blood flow.

One report suggests that spontaneous ventricular fibrillation occurs less frequently if 5% CO_2 is used in place of no CO_2 for artificial respiration. And one author claims that spontaneous ventilation was not associated with a higher incidence of fibrillation than other workers found using controlled respiration, although the majority of reports strongly implicate the depressed spontaneous ventilation as disposing to fibrillation. It has been shown that the respiratory center at 25–27° C. responds to increased pCO_2 , but this response should not be termed "normal" for two reasons: (1) the response of the control dogs to CO_2 at 37° C. was only $\frac{1}{3}$ of the "normal" expected response. (2) The response at 25–27° C. was given as a percentage of the already doubly depressed ventilation at that temperature. Similarly, it seems incorrect to state that low pH and high pCO_2 are an attempt to compensate for the depressant effect of temperature on the respiratory center — when actually they are the result of this depression.

The use of high concentrations of CO_2 to induce hypothermia in rodents has been amply confirmed. These non-hibernators appear usually to develop cardiac arrest rather than ventricular fibrillation if confined in a cold closed bottle where CO_2 builds up to about 16%. On the other hand one group suggests that the optimal pH in hypothermia is 7.5. Thus no agreement seems possible at the present time about the proper values of pCO_2 , pH and CO_2 content during hypothermia. The difficulty is partly that some workers merely cool and rewarm unoperated animals, where others are concerned with cardiac operations during the cold state. The awake poikilothermic dog is reported to keep a nearly constant arterial pH down to 30° C. pCO_2 (calculated from the data) in the only animal reported (Keller, fig. 13) is also very nearly constant, being 26 mm. at 38° C. and 32 mm. at 30° C. It is hoped that more respiratory studies will be done on these interesting poikilothermic animals.

Lung function during hypothermia is little altered. Earlier reports suggesting a limitation of diffusion for oxygen or CO_2 appear to be incorrect. Distribution and diffusion functions are well maintained as low as 20° C. in dogs, and the only major change in the lung appears to be a dilatation of the anatomic dead space. The occurrence of pulmonary edema in the warming period has been noted occasionally and needs investigation. It might especially be anticipated after surgical correction of right to left shunts or pulmonary stenosis.

Renal. A primary effect of hypothermia appears to be depression of distal tubular excretion and reabsorption. While sodium and water excretion are unimpaired, potassium excretion is reported by most workers to fall during hypothermia. Antidiuretic hormone is not capable of inhibiting the water loss. Both blood flow and filtration rate return only about $\frac{2}{3}$ to normal immediately on re-warming, but are normal within 24 hrs. A temperature of 25–27° C. is reported to

protect the kidney partially to 2 hours of ischemia. Creatinine and ammonia production are drastically reduced at low temperatures.

Endocrine. Hypothermia appears capable of largely blocking the usual adrenal response to trauma. ACTH, 17-hydroxycorticosteroids and corticoids are all greatly depressed at 25–28° C. Epinephrine output is reduced 10-fold at 26° C. and 100-fold at 21° C. Although the current tests for these substances are somewhat gross, the major changes reported seem significant. One discussant felt that the adrenal cortical response to ACTH was not completely blocked above 25° C. It is suggested that hypothermia patients do not show the usual postoperative endocrine stress pattern of overshooting the normal levels of corticoids. Patients who cannot summon an endocrine response to trauma do very poorly, and it may be that some of the immediate postoperative circulatory difficulties in patients following hypothermia may be related to this depression of adrenal response. For the most part, however, it appears to be a welcome depression of the occasionally excessive postoperative endocrine reaction.

The endocrines appear to play a significant role in natural hibernation. The adrenal and thyroid involute before hibernation, and recent evidence (Brewster *et al.*, *Circulation*, Jan. 56) (not presented at the conference) suggests that the basal metabolism raising effect of thyroxine requires the presence of circulating epinephrine. This provides the needed link in the nervous system control of heat production in hibernators.

Electrolytes. It now seems that during hypothermia tissues do not lose potassium as was formerly thought, but more than likely they actually take up potassium. This was found true in the heart both with general hypothermia, and with the perfusion of cold blood into one coronary artery. Hyperventilation at 37° may depress serum potassium 30%, whereas during hypothermia the depression from hyperventilation is given as 15%. This of course depends upon the vigor of the ventilation. Rise in serum potassium is promoted by hypoventilation, shivering, glycogen breakdown and increased metabolism. All but one investigator found calcium to rise as temperature fell. Magnesium in hibernators is said to rise 50%. Other electrolyte changes seem insignificant.

REVIEW AND APPRAISAL OF PARTS I AND II

STEVEN M. HORVATH

It is the reviewer's impression that while valuable information on the hypothermic state has been presented in this monograph, there is considerable difficulty in evaluating many of the contributions. Much of the confusion is the result of widely divergent techniques and conceptual approaches. As Dr. Frank Fremont-Smith has often said during the Macy Conferences, "Have you duplicated the experiment? Haven't you really performed another experiment?" These statements apply to most of the experiments on hypothermia.

The variables that have existed in the experimental and applied aspects of hypothermia can be summarized as follows:

- I. Physiological condition of the experimental animal*
 - A. Presence or absence of abnormal physiological states, such as cardiac or vascular defects, hepatic damage, etc.
 - B. Nutritional status
 - C. Intact animals or surgically induced variations, i.e., open chest preparations, catheterizations, exposed vessels, etc.
- II. Cooling procedures
 - A. Rate of depression of body temperature
 - B. Control of protective mechanisms against reduction of body temperature, (e.g. presence or absence of shivering)
 - C. Depth to which cooling is carried
 1. Surgical hypothermia (28° – 26° C.)
 2. Experimental hypothermia (down to -4° – -6° C.)
 - D. Duration of reduced body temperatures
 - E. Stability of reduced body temperatures both in terms of time and extent
- III. Ventilation of the organism
 - A. Spontaneous vs. controlled
 - B. Adequacy of ventilation
- IV. Rewarming and resuscitation of the hypothermic animal
 - A. Spontaneous (slow) vs. artificial (slow or fast)
 - B. Local vs. general
- V. Expression of the results obtained
 - A. Use of the rectal temperature as the criterion of the thermal state
 - B. Linear vs. non-linear temperature-dependent relationships
 - C. Interreaction (relationship?) of cellular activity to organ activity to activity of the total organism

Our concepts of the degree of hypothermia tolerated by the homoiothermic animal received a rude shock from the experiments of Andjus and Smith and of Gol-

* This term is being used here to refer to all mammalian forms utilized, i.e., man, monkey, dog, rat, etc.

lan. The successful resuscitation of animals whose body temperatures were lowered to 0° C. or below indicated that prior impressions of the lethal effects of lesser hypothermic levels (16°–24° C.) need re-evaluation. Furthermore, the procedures by which such extreme lowering of the body temperature were accomplished and the relative lack of such complications as ventricular fibrillation, suggest that there may be some misconceptions concerning the physiological mechanisms being invoked to account for the untoward incidences observed in animals subjected to mild hypothermia.

Problems which now require clarification have to do with the development of acidosis and electrolyte shifts, the use of forced (abnormal) ventilation with and without oxygen and/or oxygen and carbon dioxide mixtures. The inability of the discussants to come to a meeting of minds on these aspects of hypothermia was evident. It may be partially due to the inability to control the variables mentioned earlier or to the fact that the physiological adjustments by the organism were being interfered with to varying degrees. For example, the supposition by certain investigators that animals with low body temperatures should be ventilated with small quantities of air similar in volume and of the same or different composition to those seen when they were normothermic hardly seems valid. It is obvious from the data presented by others that the ventilatory requirements of the hypothermic animal are not those of the normothermic. Furthermore, the lack of agreement among different investigators regarding the significance of the electrolyte shifts seems to be related, in part, to their employment of arbitrary ventilatory patterns. The ultimate decision regarding the importance of electrolyte alterations would seem to rest in the demonstration of their role in animals subjected to the Andjus procedure where complete cardiac and respiratory arrests were present for appreciable periods of time.

The fact that the Andjus animal can be successfully resuscitated by applying localized heat to the thoracic area suggests that these animals may go through physiological adjustments similar to those exhibited by the poikilothermic animals discussed by Lyman and Chatfield. The biochemical and physiological capacities of this cooled heart muscle require extensive investigation. Brooks and Hoffman in their discussions have made a step, but only a step, toward providing us with this information. The difficulties inherent in this task are evident from their analysis of our present stage of knowledge.

The studies reported on the circulation of the cooled intact animal appear to be complicated to more than the usual degree by variables in technique and experimental approach. The major complicating factor in the study of the circulation is the development of ventricular fibrillation. Since the factors conducive to ventricular fibrillation are many and varied it would appear that further study with Andjus' and Smith's preparation should provide some important clues.

Reduction of the body temperature results in certain straightforward modifications of basal functioning, probably primarily related to the reduction of the metabolic level and the influence of temperature on the basic enzymatic reactions. Brown, Fuhrman, Brauer, and Gray have presented suggestive evidence of the value of considering the relationship of temperature to enzyme-substrate interreaction. While information on isolated systems can provide valuable information it

must be remembered that in the hypothermic animal the connecting link between these systems, the circulation, has also been markedly altered. Therefore, the imbalance of various metabolic chains in one system may not be transmitted properly to another system to produce either a favorable or deleterious secondary response. This emphasizes the intimate interrelationships of the various portions of the central nervous system and the products of the endocrine glands upon the response of the total organism. Studies striving to demonstrate this interrelation should prove to be most fruitful in clarifying the physiological processes inherent in the hypothermic state.

There seems to be one point of agreement among all investigators, namely that there is a decrease in the oxygen consumption in all systems of the organism in hypothermia and that this decrease may induce other effects which at the present time have not been completely evaluated.

PART III

HYPOTHERMIA AND THE PHYSIOLOGY OF CARDIAC EXCITABILITY*

CHANDLER McC. BROOKS

Function of the heart and responses of the heart to impinging influence involve two processes: (1) The origin and propagation of excitation in the heart and (2) the contractile response initiated by the propagated membrane depolarization. Cooling of the heart affects both these responses. Thus attainment of an understanding of the changes produced in hypothermia requires an analysis of the reactions involved in each of the two processes.

In this present day when action potentials are taken as a criterion of response, excitability studies tend to be confined to the determination of factors involved in initiation of a propagated action potential. The mechanical response should not be forgotten, however, and unless one considers how the membrane change and ionic fluxes involved in the excitatory process are related to the initiation of the contractile process, a complete study of cardiac excitability has not been made. The term excitability is usually defined as the ability of a tissue to detect or be affected by a stimulus to such a degree that a response ensues.

In surveying studies of cardiac excitability it seems advisable to consider: first, the present concept of the excitatory process; second, the intrinsic origin and propagation of excitation in the heart; third, the testing of excitability by the use of applied stimuli; and finally, the initiation of contraction by the excitatory process.

THE EXCITATORY PROCESS

To understand how cooling might affect the excitability of a cell one must have some concept of the excitatory process. The cardiac cell membrane is polarized and the transmembrane or resting potential is approximately 90 mv. Metabolic activity of the membrane establishes a specific partition of ions (fig. 1). Excitation occurs when the membrane is depolarized and undergoes an actual reversal of polarity. Applied current flow (cathodal) or electrotonic current flow, generated by spontaneous depolarization of pacemaker cells, in stimulating tissues does not in itself depolarize the cell membrane completely but merely reduces the membrane potential to such a degree that a regenerative process (intrinsic depolarizing reaction) is initiated (Hodgkin and Huxley, 1952). This completes depolarization and brings about the overshoot or reversal phase of the action potential. The depolarization and overshoot are associated with an influx of Na^+ as membrane permeability changes on excitation.

This initial phase, the depolarization and Na^+ flux, is followed by a repolarization of the cell and recovery of excitability which is lost during the period of re-

* Original work mentioned was supported in part by a grant from the Life Insurance Medical Research Fund.

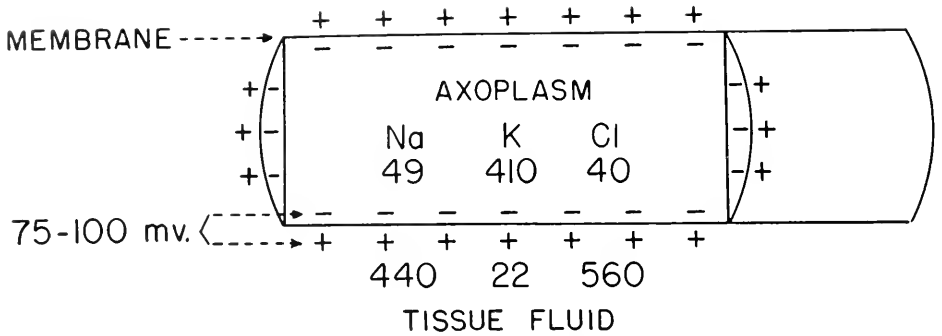


FIG. 1.—Distribution of ions between the extracellular and intracellular fluid. Squid giant axon. (Redrawn from Eccles—*The Neurophysiological Basis of Mind*, The Clarendon Press, 1953.)

versal and depolarization. It is reasonably certain that the downswing of the action potential and repolarization of the membrane involve an increase in membrane resistance, a return of sodium impermeability, an outward flux of K^+ and activity of an ion exchange pump. In the subsequent paper evidence will be presented which indicates that repolarization process consists of more than one phase and is far from a simple process. Additional information concerning excitatory processes can be found elsewhere (Hodgkin, Huxley and Katz, 1952; Brooks *et al.*, 1955).

In conclusion it can be said that excitation of the heart involves numerous complex processes which might be affected differently by cooling. Furthermore there is an excitability cycle. Since excitation involves depolarization, the heart cannot be reexcited following one stimulation or an intrinsically originating beat until repolarization has again occurred. One might also expect excitability of the heart to be subnormal or at least abnormal during the phase of progressive repolarization and this is the case.

The next questions which arise are: how stable is the cardiac cell membrane normally; how easily and by what process can excitation occur; how does hypothermia modify these reactions?

THE INTRINSIC ORIGIN AND PROPAGATION OF EXCITATION

The heart is comprised of potentially autonomic units which are dominated by a pacemaker. This pacemaker initiates an excitatory process which drives other units at a faster rate than that which would be established intrinsically. Two different phenomena are involved in establishment of the cycle of excitability changes and mechanical events which typify cardiac activity. These are first, the intrinsic excitatory process in pacemakers and second, the excitation of other portions of cardiac tissue by activity beginning in a pacemaker.

Hypothermia and heart rate. Knowledge that cooling affects the intrinsic excitatory process was obtained first from studies of heart rate. There have been many determinations made of the effects on heart rate of cooling the body and the heart (Badeer, 1955; Brooks *et al.*, 1955). When the skin is cooled, when muscles become active and when epinephrine is released from the adrenal medulla on cooling of the body (Cannon, 1932) the heart is caused to accelerate by the resulting

neurochemical reactions. Section of the cardiac nerves and denervation of the adrenals greatly lessens this response. As the heart itself is cooled the rate falls. Within certain critical ranges a direct relationship is found between rate and heart temperature but outside these limits expression of relationships are curvilinear (Knowlton and Starling, 1912; Badeer, 1951; Hegnauer, 1952; Kao *et al.*, 1955). Asystole may occur when the heart is cooled to 20° C. in some instances but usually the rate falls to about 20 per minute at 15°–17° C. and the heart stops beating regularly below 13° C., although a few sporadic beats may occur down to 8° C. Spontaneous arrhythmias are a common consequence of cooling (Brooks *et al.*, 1955) and fibrillation may occur before asystole.

Changes in cardiac output tend to parallel those in rate. Following a brief initial augmentation, probably due to shivering and autonomic compensatory activity, there is a progressive drop in output with cooling. In large dogs the output remains at approximately 1.5 to 1.23 liters per minute between heart temperatures of 38° to 28° C. but below that level it falls sharply and at 23.5° C. is only 0.65 liters per minute (Kao *et al.*, 1955). At these low temperatures, however, the heart pumps more blood in relation to O₂ consumption of the body. It can be concluded therefore, that unless the oxygen requirements of brain or other special tissues remain higher and do not change proportionately with those of the rest of the body, the drops in heart rate and output do not cause a serious hypoxia until arrhythmias or irregularities of beat occur at very low heart temperatures (Brooks *et al.*, 1955).

The effect of cooling on pacemaker action. In early studies of the locus of the pacemaker (Eyster and Meek, 1921) it was found that cooling the SA node so depressed its activity that pacemaker action was assumed by the uncooled regions of the heart. Furthermore it was shown that localized heating of regions other than the sinus tended to establish an ectopic pacemaker (Eyster and Meek, 1921; Scott and Reed, 1951). Thus temperature changes definitely can influence origin of the excitatory process.

Recent studies indicate that the pacemaker is that region of the heart which possesses the most unstable membrane. As soon as pacemaker cells repolarize a gradual spontaneous depolarization begins as indicated by the presence of a pre-potential (Erlanger, 1913; Rijlant, 1928; Draper and Weidmann, 1951). Little is known about the nature of the processes involved; slow depolarization might be due to positive actions tending to depolarize or to a deficiency of stabilizing or polarizing forces (Brooks *et al.*, 1955). One thing is certain and that is that in non-pacemaker tissues normal excitability is recovered long before initiation of a beat begins and in non-pacemaker tissues no potential change similar to that occurring in pacemakers is seen preliminary to the propagated action potential.

One of the best methods of studying events in the pacemaker which produce a propagated response is that of intracellular recording (Draper and Weidmann, 1951; Brady and Hecht, 1954; Hutter and Trautwein, 1955). The Q_{10} of the slow diastolic depolarization of the pacemaker which originates a beat is 5. The Q_{10} of other phases of the action potential have been found to be much lower (Trautwein, 1953; Coraboeuf and Weidmann, 1954). In the subsequent paper on cellular potentials the effects of cold will be more adequately discussed.

Hypothermia and the propagation of excitation. Conduction of an impulse

is merely due to propagation of the excitatory process. When the pacemaker tissue cells depolarize or reverse polarity of their membranes, the proximity of a negatively charged surface to normal positively charged surrounding tissue sets up a flow of current. This electrotonic current flow tends to depolarize the normal tissue through which it passes, thus touching off a regenerative process. If the tissues have normal excitability and the voltage difference giving rise to current flow is great enough, excitation occurs.

It is generally conceded that cooling slows conduction and eventually decreases the excitability of tissues. The question thus arises as to the adequacy of the normal means of propagation. It is estimated that in nerves the strength of the excitatory process involved in conduction of an impulse is three to ten times the threshold requirement (Hodgkin, 1937; Bishop, 1951). No similar direct determinations have been made in the heart but the older experiments of Junkmann, 1925 and Witz, 1938, indicate a large factor of reserve in the normal propagating mechanism.

In nerve and the central nervous system cooling produces a very great increase in the duration and a slight increase in the amplitude of action potentials; even the action potentials of individual fibers are thus modified (see Brooks, Koizumi, and Malcolm, 1955). In the heart, duration of the surface-recorded and transmembrane potentials is a function of heart rate and/or heart temperature. Schütz (1936) has reported that repolarization in the cooled heart is completed before recovery of normal excitability following origin of a beat. Cooling prolongs the duration of electrical activity and the coefficient for the duration of the monophasic action potential was found to be 2.2 for every 10° C. (Lepeschkin, 1951). The relationship between the effect of cooling on repolarization and recovery of excitability has not as yet been worked out.

Progressive cooling of the heart ultimately causes a reduction in height (voltage) of the action potential. Initially, however, the R, S, and T waves increase in amplitude (Hamilton *et al.*, 1937; Decker, 1939; Lange *et al.*, 1949). This increase in voltage has been explained on the basis of slowed conduction (see Lepeschkin, 1951). Studies of transmembrane action potentials of the heart cells have shown that on cooling of the tissue the height of the potentials and the extent of the overshoot is increased until a critical temperature is reached (25° C.) below which depression occurs in a progressive fashion. The subsequent paper will deal more fully with the effects of cold on the voltage of action potentials. These voltage changes do relate to the strength of the excitatory process in hypothermia. Additional information concerning changes produced in the electrogram and electrocardiogram of man and animals as a result of local or general cooling of the heart is given by Lepeschkin (1951).

The fact that cooling slows conduction indicates that the process of excitatory depolarization is slowed as are repolarizing reactions. The slowing of A-V conduction (P-R interval increase) and conduction in the ventricle (Q-S interval increase) is linear between 40° and 16° C. (Lutz, 1948). Eventually A-V block develops at very low temperatures. Slowed ascent of the monophasic action potential has been reported (Schütz, 1936; Decker, 1940) and in studies of individual cellular reactions it has been found that the rate of rise of the transmembrane action potential is slowed and the process is prolonged. A longer time requirement for

reduction of membrane potential to the critical value by electrotonic current flow and a slowing of the regenerative process which completes the reversal of membrane polarity explain slowing of conduction in hypothermia.

The same processes are involved in propagation of excitation whether the impulse originates in the activity of a pacemaker or in an applied stimulus. In discussing the initiation of activity by external stimuli as excitability is tested, no further mention need be made of how the excitatory process spreads throughout the heart.

DETERMINATION OF THE EFFECT OF HYPOTHERMIA ON CARDIAC EXCITABILITY BY THE USE OF TESTING STIMULI

Methods. Relatively simple methods can be employed to study changes in excitability which occur during the cardiac cycle and to determine the effect of hypothermia on excitability. The major difficulties involved are: (a) the accurate placement of testing stimuli at specific intervals of the cycle, and (b) the estimation of the parameters of the effective stimuli so that the quantitation of work performance required to elicit a response is known.

Stimulation of the heart requires use of electrodes. Chronically implanted electrodes and thermocouples presumably provide the most ideal conditions for such work. In most instances, however, acute experiments have been performed, and the results from these are similar to those obtained from chronic preparations, provided the chest is closed and the heart has a relatively normal surround.

Accurate placement of test stimuli has been accomplished by triggering the testing stimulator from the R wave of the electrocardiogram or electrogram and placement of stimuli at specific times after the peak or the beginning of the recorded R wave. An easier and in some ways a more satisfactory method is to supplant action of the intrinsic pacemaker with a driving stimulator. The testing stimuli can then be placed with reference to the arrival of the driving stimulus.

Phases of the excitability cycle identified by testing stimuli. Study of the cyclic changes in cardiac excitability by this method has revealed many interesting facts. If the cycle is considered to begin with the initiation of propagated activity (the Q wave of the electrogram) it can be seen that:

(a) There is a refractory period which in total duration is approximately identical with the phases of depolarization and repolarization or the Q-T interval of the electrogram. This refractory period can be subdivided into (1) an early or absolutely refractory period associated with depolarization and the early or slow phase of repolarization and (2) a relative refractory period during which the terminal or quickly occurring repolarization occurs. The boundary between the absolute and relative refractory period is not well defined because it is determined principally by the efficacy of the testing stimuli (fig. 2-A).

(b) The refractory period is likewise an irresponsive period in that a normally propagated response cannot occur until some time after completion of full repolarization. The latency between application of testing stimuli and appearance of propagated excitation increases as a stimulus is applied earlier and earlier in the refractory period. Consequently, an effective stimulus, to act on the heart during

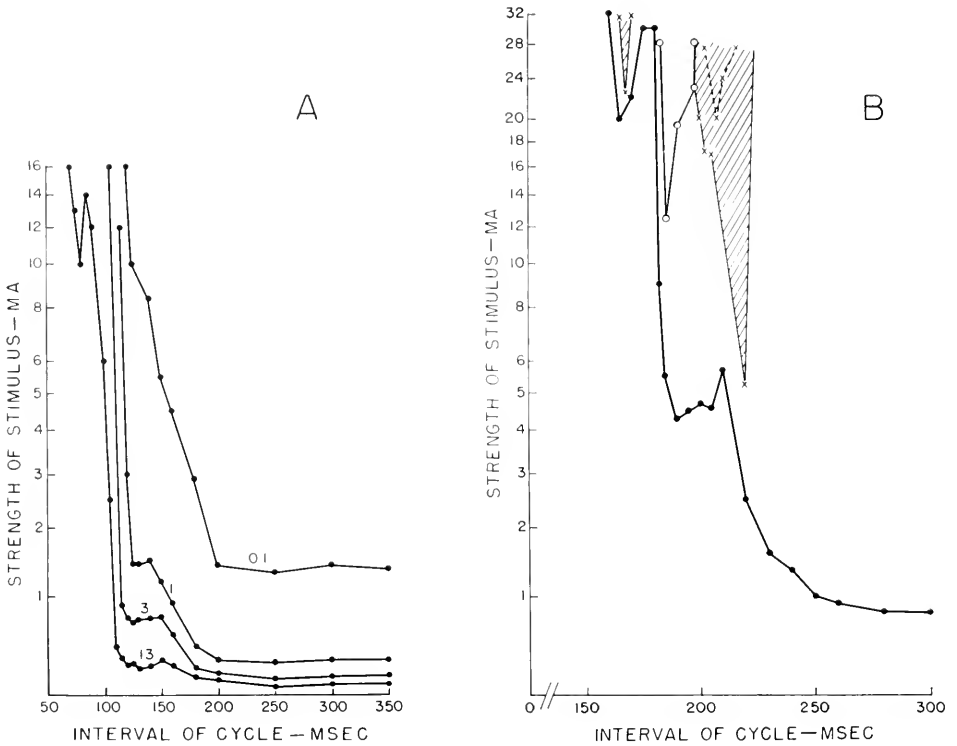


FIG. 2.—(A) Strength-interval curves obtained with stimuli of different durations, showing boundaries of absolute and total refractory periods as defined by each. Note early and late dips in 13 msec. test shock curve. (B) Strength-interval curve obtained from cat ventricle showing dips and periods of vulnerability to fibrillation. ∇ = multiple extrasystoles; ∇ = fibrillation; \odot = no response at all to very strong stimuli. (From Amer. J. Physiol. 163: 469, 1950; 167: 88, 1951.)

the refractory period, must have an effect which can persist as a local excitatory state, a local non-propagated response or one so slowly propagated that its travel cannot be recorded by means usually employed (fig. 3).

(c) Recovery of excitability is a far from simple process. Testing of the change in excitability associated with the terminal quick phase of repolarization shows that at certain specific intervals a degree of recovery of excitability is attained which is not sustained. Usually there are two such intervals or "dips" in the strength-interval curves of the auricle and ventricle (fig. 2). Repolarization is often followed by a phase of supernormality (fig. 4). There is thus an oscillation or fluctuation shown in curves expressing recovery of normal excitability.

(d) The heart is vulnerable to fibrillation by single strong electrical stimuli at these "dip" intervals. Stimuli of progressively increasing strength produce extrasystoles, then multiple extrasystoles, and, at still higher strengths, actual fibrillation. This occurs in both auricle and ventricle when stimuli are applied at these

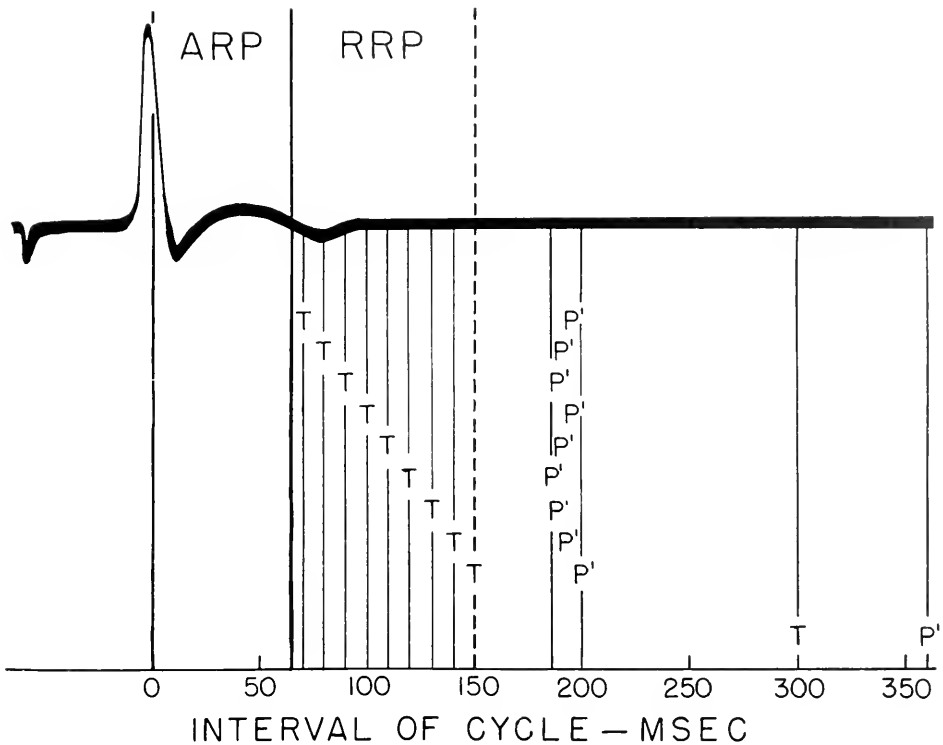


FIG. 3.—Diagram of dog auricular electrogram showing boundaries of absolute and relative refractory periods and the latency between application of testing stimuli (T) placed at various periods of the cycle and the response (P^1). (From Brooks *et al.*, 1955.)

critical phases of the refractory period but at no other intervals of the cycle (fig. 2-B). It should be pointed out that threshold stimuli produce, during the refractory or irresponsive period, an effect which ultimately gives rise to a single extrasystole. Stronger stimuli placed at these specific intervals (the vulnerable periods) have an effect which eventuates in fibrillation. The persisting excitatory processes in the two cases may be different.

Fibrillation as a response to excitation. Fibrillation can be considered one type of response resulting from stimulation of the heart. Fibrillation thresholds and the effects of lowering heart temperature on the tendency to fibrillate can be determined by methods described. In hearts cooled to a critical temperature of 20°–25° C. arrhythmias may develop spontaneously (Talbot, 1941; Alexander, 1946; Hegnauer and Covino, 1955) but they are more likely to result if mechanical (Hegnauer *et al.*, 1951) or electrical (Pinkston *et al.*, 1953) stimulation occurs.

Fibrillation is a continuous disorganized activity of myocardial cells. A reasonable assumption to make is that any influence which creates abnormally great dissimilarities of cellular excitabilities, either throughout the entire heart or in a local region, would favor establishment of fibrillation by favoring escape of certain cells from dominance by the pacemaker or by permitting a saltatory type of conduction over the heart. By saltatory conduction is meant stimulation of cells at some dis-

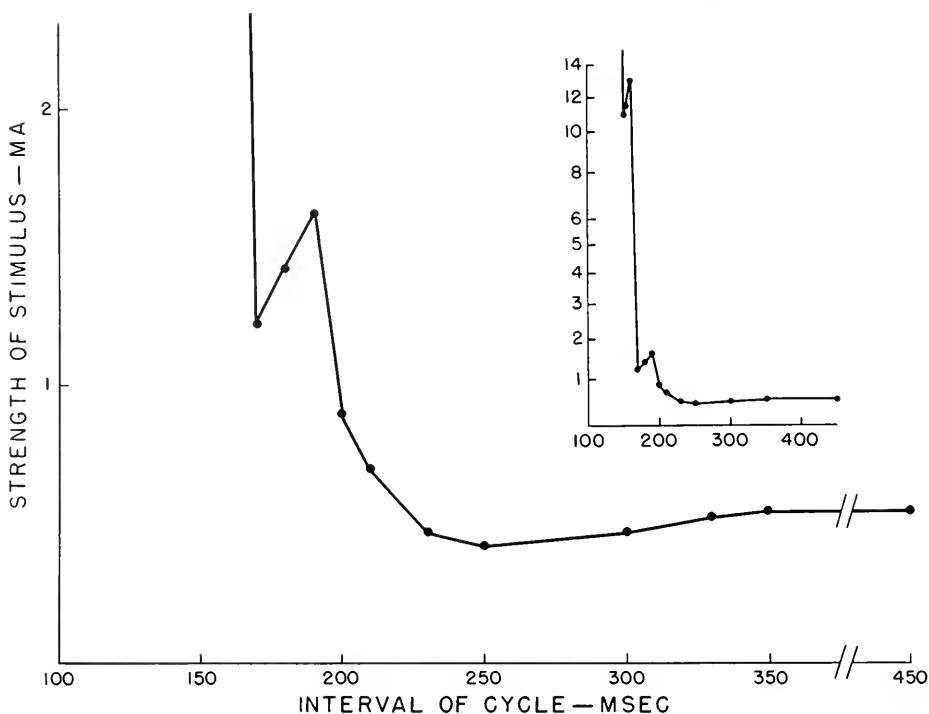


FIG. 4.—Supernormality. Insert shows total ventricular strength-interval curve from which enlarged segment was taken. (From Brooks *et al.*, 1955.)

tance from rather than adjacent to the boundary of the depolarized area, thus touching off ectopic foci of excitation and disorganizing heart action (fig. 5, A and B). Localized injury or depression such as might be caused by local application of cold also creates a condition favorable to saltatory conduction and disorganized progression of the excitatory process (fig. 5C).

The effects of hypothermia on the processes identifiable by the methods described. In studying the effects of drugs, cooling and heating on organs such as the heart it is necessary to differentiate between direct effects and those of a secondary order.

(a) *Indirect effects.* (1) Changes in heart rate result in modification of the duration of certain phases of the action potential and the refractory period (Siebens *et al.*, 1951). Changes in rate modify the Q-T interval, the duration of the absolute refractory period and the "plateau phase" of the transmembrane action potential but have little effect on the late or quick phase of repolarization and consequently do not modify the duration of the relatively refractory period (fig. 6). If the heart is permitted to accelerate or slow during testing, the effects of temperature *per se* on the duration of phases of refractoriness, latency of response, etc., must be corrected for effects resulting merely from changes in heart rate.

(2) Apparent effects of hypothermia on the heart might be merely the result of slowing ion diffusion rates. Action of cold on ion partition and ionic flux across cell membranes must be considered as an effect of hypothermia on the heart since

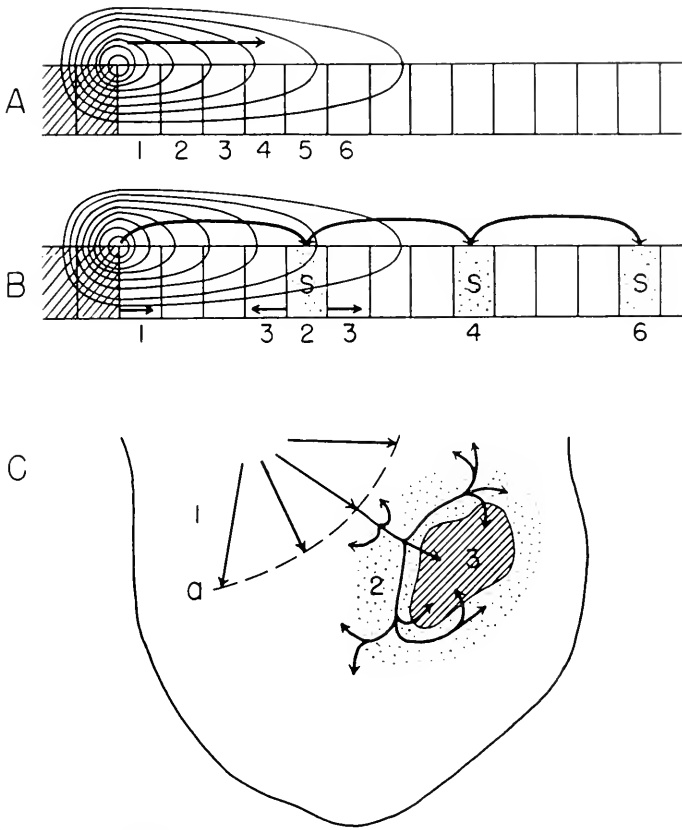


FIG. 5.—Diagram of possible saltatory conduction. (A) Normal propagation through cell adjacent to sink (depolarized area). (B) Jump from sink to most excitable cells (S). Arrows indicate direction of spread of excitation. (C) Conduction in normal tissue: (1) with a jump into tissue rendered hyperexcitable by current of injury, (2) slowed conduction in depressed area, (3) arrows show possible course of impulse propagation.

ion distribution and transport of ions across cardiac cell membranes is associated with excitability and excitation.

(3) The normal heart is supplied by an autonomic innervation and is subject to action of circulating epinephrine and nor-epinephrine. If the reactions of the body normally associated with maintenance of homeostasis are permitted to occur in production of hypothermia, the actions of these agents on the heart must be differentiated from the direct effects of cooling. It is known that autonomic effectors (nerves and mediators) affect repolarization of cells and have biphasic effects on excitability and vulnerability to fibrillation (fig. 7). (See Brooks *et al.*, 1955.)

(b) *Direct effects.* In considering direct action of hypothermy on the irritability of the heart one must differentiate between regional or asymmetrical cooling and generalized or uniform hypothermia of the cardiac tissue.

(1) Cooling one region more than another is known to establish localized electrotonic current flow (Granit, 1955) which has at least a subthreshold excitatory

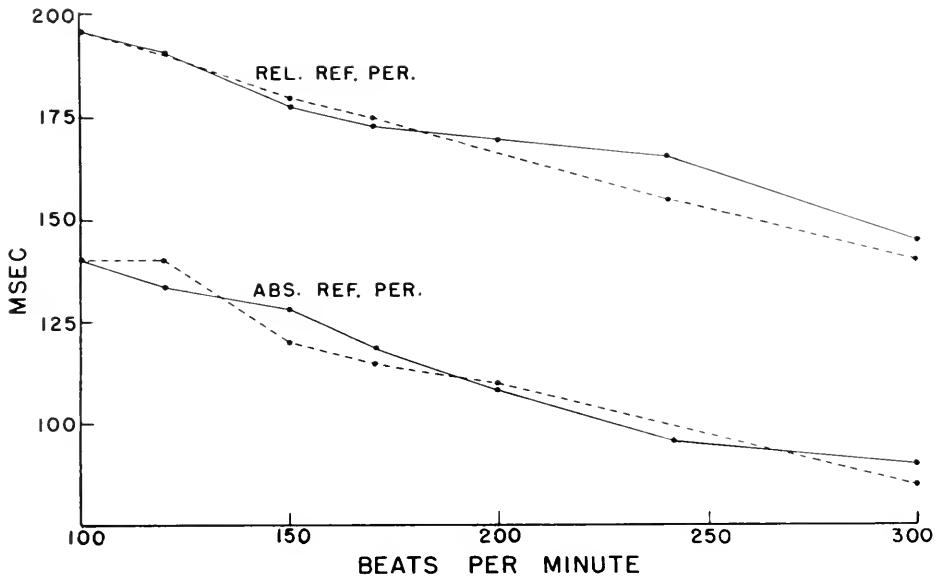


FIG. 6.—Constancy of duration of relative refractory period and change in absolute refractory period as dog ventricular rate is increased. Ventricle driven directly (—), auricular drive of ventricle (-----).

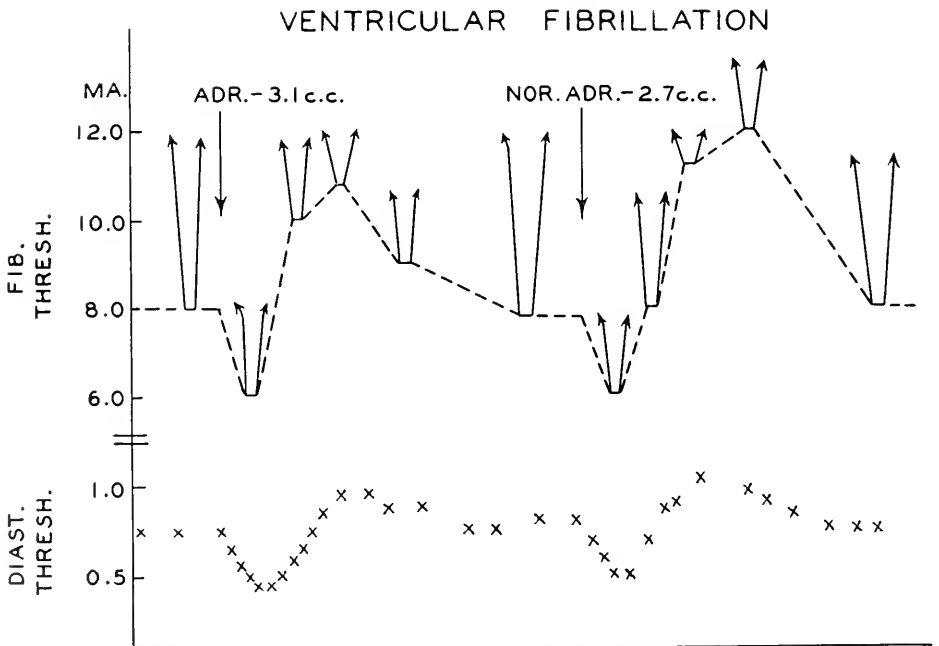


FIG. 7.—Changes in diastolic threshold and fibrillation thresholds of the dog ventricle following injections of adrenalin and nor-adrenalin (1:25,000).

action. Such localized excitatory influences predispose to disorganization of normal propagation of impulses, increase vulnerability to fibrillation, or may actually create arrhythmias.

(2) Uniform cooling has a selective action on various processes and phases of depolarization and repolarization. When corrections are made for changes in heart rate or rate changes are prevented, it is found that cooling does have definite effects but some response potentialities are affected more than others.

Thresholds to stimuli applied during diastole are not markedly affected over a wide range of temperature change. As an example, in one experiment threshold in the dog ventricle was 0.13 ma. at 39.4° C. and 0.17 ma. at 26.2° C. while for the auricle the threshold stimulus intensity was 0.19 ma. at 39.7° and 0.18 ma. at 26.7° C. All changes observed in the 30 animals studied were usually in hundredths of a milliamperere within the temperature range mentioned (see Brooks *et al.*, 1955). Cooling below this level did cause a greater decrease in excitability and it has been reported that on rewarming of the heart there is a lag in recovery of normal excitability particularly if very low temperatures are reached.

The testing methods described show that strength-interval curves shift in position as the heart is cooled, indicating a slowed recovery of excitability, but at least under some conditions of induced hypothermia no consistent change in amplitude or duration of the dips has been observed. Others have found a marked increase in depth of the early dip (Hegnauer and Covino, 1955). This relatively greater temporary supernormality might contribute to vulnerability but the effect of cooling on fibrillation thresholds and duration of the vulnerable periods has not as yet been determined.

It is undeniable that the cooled heart is more susceptible to fibrillation. Auricular and, less frequently, ventricular arrhythmias and fibrillation develop spontaneously in both anesthetized and unanesthetized hypothermic man and other animals. Mechanical stimulation of the cooled heart by indwelling catheters tends to produce fibrillation and the general impression is that stimulation by electrical pulses is more likely to result in fibrillation of the cold than of the normally warm heart. Susceptibility of the heart to fibrillation cannot readily be explained on the basis of changes in excitability but there are other changes which should be considered.

Cooling slows conduction. This is explained by the change in time of rise of the action potential. Since the upswing of the action potential is due to the inward flux of Na^+ one can conclude that cooling slows the regenerative process which permits the sodium influx and/or slows the influx itself (see chapter on cell potentials, Hoffman). A change in conduction velocity theoretically could contribute to a disorganization of cardiac action.

Shift of the strength-interval curve indicates a prolongation of refractoriness in the cooled heart. As stated previously the action potential is prolonged as repolarization processes are slowed. It may be of significance that the change in duration of the absolute refractory period is greater than the change in the relative refractory phase (Pinkston, 1956). This indicates that certain of the processes involved in recovery of depolarized cardiac tissue may be more susceptible to cooling than are others. One of the most striking observations is that of Schütz (1936) that refrac-

toriness is prolonged out of proportion to the lengthening of the refractory period. Here again is evidence of a selectivity of action of cold on cardiac processes.

One final point worthy of mention in this discussion of cardiac-excitability testing is that at low temperatures the heart is difficult to drive. It becomes unable to follow stimuli applied at a faster rate than that of its own intrinsic pacemaker. This may be due to an inability to conduct impulses more frequently than they are originated. The specialized conducting system due to a faster rate of spike rise can conduct faster than undifferentiated ventricular tissues but its slower rate of repolarization (see chapter on cell potentials, Hoffman) may determine the limit to heart rate acceleration in the hypothermic heart. It has been reported that accelerated drive of the hypothermic heart (Berne, 1954) decreases the effectiveness of its mechanical action.

Many of these points mentioned in this introductory presentation will be amplified and more precise information will be given by subsequent speakers who have been interested in this same problem—the effect of hypothermia on cardiac irritability.

HYPOTHERMIA AND THE INITIATION OF A CONTRACTILE RESPONSE

Consideration of the effect of hypothermia on mechanical contraction of the heart may be considered within the province of the topic assigned because it cannot be denied that contraction is the most significant element of the response of the heart to intrinsic or applied stimuli.

The propagated electrical response normally initiates a contractile process. It appears that contraction follows the propagated depolarization and repolarization with a certain latency. Very early in the cycle a normally propagated action potential can be evoked dissociated from any significant contraction. Evidently ability to contract is regained more slowly than the ability to be excited and to conduct an impulse (fig. 8).

The very early conducted impulse, though it has no immediate visible effect on a contractile mechanism, does potentiate subsequent contractions when they occur. The duration of this potentiating action has been determined (Hoffman *et al.*, 1956; Siebens *et al.*, 1956) but its nature is not known. The possible effects of temperature on this phenomenon have not been determined.

It has been demonstrated that autonomic activity associated with exposure to cold does modify the heart's contractile process. Epinephrine and accelerator nerve impulses, even though heart rate and venous return are kept constant, do cause an increase in amplitude of myocardial contractions (Wiggers, 1952). They show a steeper rise and a shorter duration.

Cooling *per se* should have some direct action on the contractile processes of muscle. In this connection it can be said that reduction of temperature is reported to increase total tension of contractions in frog skeletal muscle (Wiggers, 1949). According to Szent-Györgyi (1948) muscle contraction has a very high temperature coefficient. At 0° C. there is no contraction at all in mammalian muscle while at 16° C. contraction is maximal. Szent-Györgyi also describes some calculations of

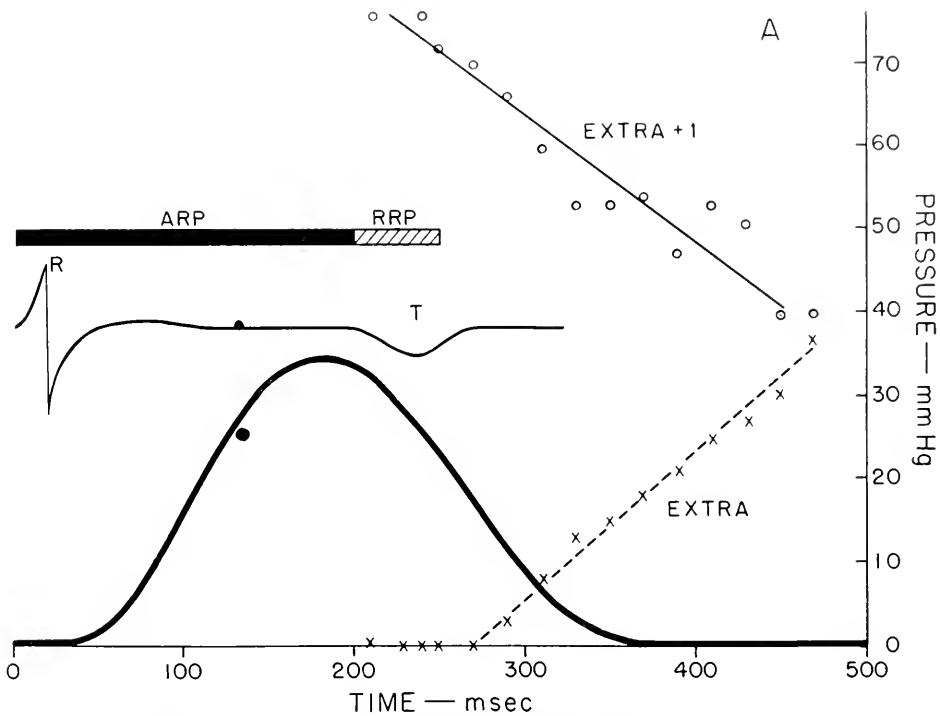


FIG. 8.—Electrogram and ventricular pressure pulse showing temporal relationships. Amplitude of pressure change in closed ventricle during an extra electrical systole at times (X) and the pressure developed by subsequent systole (Extra + 1) (O). (Brooks *et al.*, 1955.)

free energy changes in muscle and their dependence on temperature. There is a straight-line relationship between temperature and the free energy in muscle slices when contracting; at higher temperatures there is a higher energy change. Actomyosin threads behave in a similar manner. Studies of pressure pulses during hypothermia have shown that the observed decline of blood pressure is not a manifestation of myocardial failure (Berne, 1954). In arousal of hibernating mammals pulse pressure increases transiently, then declines as the heart is warmed (Lyman and Chatfield, 1955). In the cooled heart (30°C .) concentrations of the drug dinitrophenol, which decreases work capacity of the normothermic heart (39°C .), has no negative inotropic effect (Rothlin *et al.*, 1955). Other studies (Hegnauer and D'Amato, 1954) have shown that at 17°C . the work output of the heart per minute is only 7.3 per cent of the control value obtained at normal heart temperatures. The work per stroke, however, is reduced by only 50 per cent at this low temperature. Trautwein and Dudel in a series of papers (1954) showed that progressive cooling of isolated cat papillary muscle and Purkinje tissue of the dog from 38°C . to 19°C . produced the following changes. At constant rates of beat there was still a gradual prolongation of isometric contraction and relaxation. Amplitude of the isometric contraction was increased up to a maximum and then decreased progressively. Amplitude of the action potentials increased with the fall in temperature to a critical point and then decreased. Apparently no single

or simple intracellular mechanism can account for all changes in amplitude of cardiac muscle response. Certainly additional study of the effect of cooling on the contractile process and the reactions which touch off contraction when an impulse traverses the cell membrane should be carried on by those interested in hypothermia and its effect on the function of the heart.

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TEMPERATURE EFFECTS ON CARDIAC TRANSMEMBRANE POTENTIALS

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One of the major problems encountered in the use of hypothermia as an adjunct to cardiac surgery is the occurrence of either ventricular fibrillation or, somewhat less frequently, cardiac arrest. It seems likely, in light of recent investigations,^{1, 2, 3} that in most cases a direct effect of temperature on the cardiac muscle is not primarily responsible for the onset of fibrillation. To the contrary, considerable evidence indicates that respiratory depression and resulting changes in P_{CO_2} and pH of the blood are more directly linked to the production of arrhythmias.^{1, 4} In support of this proposition is the demonstration that fibrillation does not eventuate in dogs at the usual critical temperature range if ventilation is controlled and development of acidosis prevented.² Similarly, it has been shown that changes in the ventricular excitability cycle similar to those found prior to the onset of hypothermic fibrillation can be reproduced at normal body temperature by alterations in the pH of the arterial blood.¹ The possible role of hypoxia in the production of arrhythmias under hypothermic conditions is thought to be of minor importance;^{6, 7} adequate studies of the state of tissue oxygenation, however, have not been performed.

On the other hand, the importance of a direct effect of low temperature on the myocardium should not be neglected. For example, even under conditions of controlled ventilation and arterial pH there are major changes in the duration of refractoriness, the time-course of the recovery of excitability and the resting or diastolic threshold.^{2, 5} Conduction velocity is decreased by cooling as is spontaneous rhythmicity; moreover, cardiac arrest occurs during hypothermia even in the absence of major alteration of arterial pH.² Low temperature has a profound effect on the delivery of oxygen to the tissue cells by myoglobin⁹ and may contribute to the development of a metabolic acidosis in spite of artificial hyperventilation. Finally, older investigations have shown that cooling may result in a temporal dissociation between the recovery of excitability and the repolarization of the fiber membrane.¹⁰

It is difficult to dissociate any study of the effects of temperature or pH from a consideration of what may happen with respect to the common inorganic ions. In both nerve¹¹ and skeletal muscle¹² a decrease in temperature results in a loss of K from within the cell. Furthermore, it has been noted that prior to the onset of fibrillation in hypothermic dogs there are significant changes in the net fluxes of K, Ca, and H across the cardiac cell membrane.²

In view of these considerations, it seems likely that a review of the effects of temperature, ions, pH and P_{CO_2} on the membrane activity of single cardiac fibers is pertinent to this part of the Symposium. The information presented has been obtained by means of studies of the transmembrane potentials of single fibers. The advantages of this experimental approach are several. In the first place, the activity of a single unit in a multifiber preparation can be investigated without alteration of

the normal relationship between fibers. Furthermore, the absolute value of the transmembrane potentials can be determined with certainty and the time-course of potential changes during activity clearly delineated. Finally, observed changes in threshold and refractoriness can be related to alterations in the state of polarization of the fiber membrane.

Technique. The transmembrane potentials of single cardiac fibers are recorded by means of a fine glass capillary microelectrode of the type developed by Ling and Gerard.¹³ The microelectrode employed is drawn from capillary tubing to a tip diameter of less than one micron and filled with a concentrated solution of KCl (3 M) to minimize junction potentials between myoplasm and electrolyte.¹⁴ It has been demonstrated that insertion of an electrode of this size through the fiber membrane does not, of itself, result in measurable injury.^{14, 15} Although movement resulting from contraction often dislodges the tip of the microelectrode from within the fiber, in most preparations it is possible to record many cycles of activity from a single fiber without change in the magnitude or time-course of the transmembrane potential.^{15, 16} In contrast to the motoneuron of the cat spinal cord which is strongly affected by leakage of Cl from within the microelectrode,¹⁷ the resting and action potentials of single heart fibers are unchanged even after several hours of recording from the same area of the membrane.^{15, 16}

In practice the microelectrode is paired with an indifferent electrode of similar composition. The potential difference recorded when both electrodes are extracellular in position is the reference or zero potential. When the tip of the microelectrode is inserted through the membrane and into a resting fiber a potential difference of approximately 90 mv is recorded (fig. 1). The sign of this potential difference is such that the inside of the membrane is negative with respect to the outside. This potential is commonly called the resting (transmembrane) potential. With the onset of activity there is an abrupt change in the transmembrane potential which consists of a rapid depolarization and reversal of membrane polarity (inside positive with

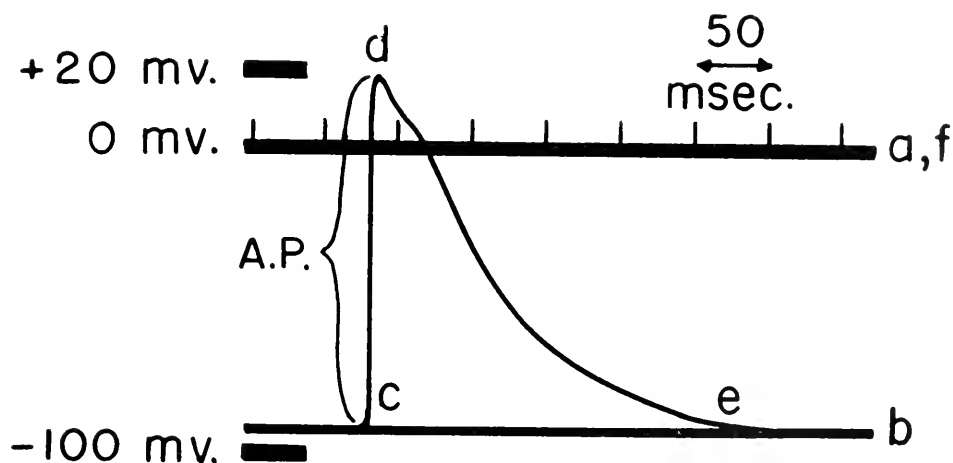


FIG. 1.—Diagrammatic record of transmembrane potentials of auricular muscle. Line a, f = zero potential; b = resting potential; c, d, e = action potential. Time and voltage calibrations shown in figure.

respect to outside) and a subsequent slower phase of repolarization which restores the normal resting potential (fig. 1). This sequence of changes is called the (transmembrane) action potential and differs in certain respects between fibers obtained from various parts of the mammalian heart.

Normal records. Typical records obtained from isolated preparations of the dog auricle, papillary muscle and Purkinje system are shown in figure 2. Certain differences between the transmembrane potentials of these three tissues are immediately apparent. The resting potentials of both auricle and ventricle, amounting to 85–90 mv,¹⁶ are somewhat smaller than that of the dog Purkinje fiber (90 mv).¹⁵ Similarly, the magnitude of the reversal or overshoot is less in the unspecialized fibers (15 mv) than in the specialized conducting system (30 mv). Most striking, however, are the differences in the time-course of repolarization revealed by the three types of fiber. In the case of the auricle, repolarization commences immediately after the upstroke of the action potential and proceeds with a relatively constant velocity to completion. Action potentials recorded from single ventricular fibers, on the other hand, reveal an initial spike (lasting 10–15 msec.) followed by a plateau during which the membrane potential remains close to zero. The plateau, in turn, terminates in an abrupt phase of repolarization. In terms of total duration, the action potential of the ventricular fiber is only 50 msec. longer than that of auricular muscle.

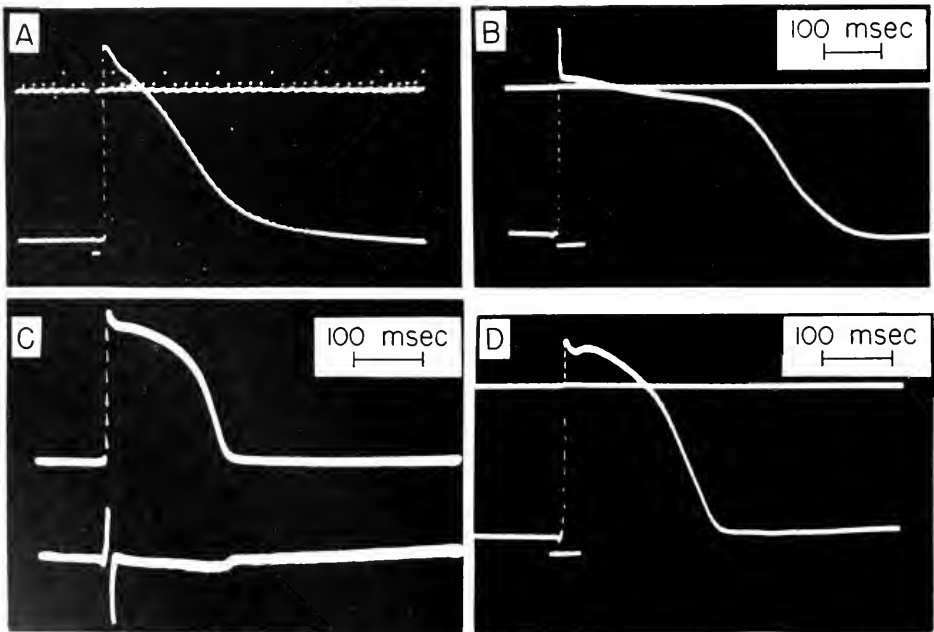


FIG. 2.—Transmembrane action potentials of single myocardial fibers. (A) Auricular action potential — time marks on upper trace in 10 and 50 msec; — 100 mv. calibration shown just below upstroke of action potential. (B) Purkinje fiber action potential, — 100 mv. calibration shown as in A. (C) Simultaneous records of transmembrane action potential (top trace) and unipolar electrogram (bottom trace) of ventricular muscle. (D) Ventricular action potential from same heart as B. Action potential upstroke retouched in all cases.

The action potential recorded from the Purkinje fiber shows a more prominent spiked reversal of polarity which lasts only a few msec., a prolonged plateau, and a phase of repolarization which is slower than that of ventricular muscle.¹⁵ While the duration of depolarization is quite similar in auricle and ventricle at similar heart rates, the Purkinje fiber action potential lasts approximately twice as long as that recorded from plain ventricular muscle.^{18, 19} This difference in action potential duration is present at both slow and rapid heart rates. One additional factor, not apparent in the figure, is the considerable difference in rising velocity of the action potentials recorded from specialized and plain cardiac muscle. The maximum rising velocity of the action potential recorded from a single Purkinje fiber amounts to 500–1000 v/sec;¹⁵ this is considerably greater than values obtained for undifferentiated ventricular fibers.¹⁹

Records obtained from isolated preparations of dog auricle and papillary muscle are similar to those obtained from the intact heart *in situ*²⁰ and are comparable to the transmembrane potentials of the intact human heart.²¹

Ionic basis of transmembrane potentials. The distribution of ions between intracellular and extracellular water is such that, in the case of cardiac muscle, the concentration ratios of the major cations between the inside (I) and outside (O) are: $K_I:K_O=30:1$; $Na_I:Na_O=1:10$;^{22, 23, 24} and $Ca_I:Ca_O=1:1$.^{25, 26} Much less is known about the actual concentrations of the intracellular anions; however, the concentration of Cl within the fiber is probably considerably less than that in the extracellular fluid.²⁷ Under resting conditions the fiber membrane is somewhat permeable to all of these ions, and during activity the net fluxes of K and Na increase considerably (see below). An explanation of the concentration gradients thus cannot depend solely on membrane impermeability.

In the case of the isolated squid giant axon¹¹ experimental evidence indicates that Na is actively extruded from within the axoplasm across the membrane by a transport mechanism referred to as the "sodium pump." This transport takes place against both the concentration and potential gradients. Such transport of positive charge from inside to outside a membrane which behaves as a double-layer capacity²⁸ would tend to create a potential difference across the membrane and thus might be responsible for the intracellular concentration of K. The magnitude of the resting transmembrane potential (-90 mv) is in accord with an activity ratio for K of approximately 30:1 and thus the distribution of K ions might be passive in nature. On the other hand anoxia and certain enzymatic inhibitors in proper concentration cause a decrease not only in the rate of Na extrusion but also a similar change in the rate at which K enters the fiber.¹¹ However, under these conditions the rate of K loss from the fiber and the magnitude of the resting transmembrane potential are not significantly altered. These results suggest that while the accumulation of K depends on metabolic activity and may be related to active Na transport the outflux of K is passive in nature.

A direct dependence of the resting potential on the K concentration gradient and outward diffusion of this ion across the membrane is suggested by the observation just mentioned. Additional evidence in support of this proposition is afforded by the demonstration that the magnitude of the resting potential is inversely propor-

tional to the log of the extracellular K concentration²⁹ but is not appreciably influenced by even a complete absence of extracellular Na.¹⁵

The membrane potential changes associated with activity are somewhat more complex in nature. It has been demonstrated that, in the squid giant axon, the upstroke of the action potential results from a specific change in the Na permeability of the membrane and a resulting inward (positive) current carried by this ion.⁴¹ Repolarization, in turn, results from a subsequent decrease in Na permeability and rise in K permeability;⁴² the resulting net outflow of positive charge carried by K ions restores the resting transmembrane potential to normal values.

In cardiac muscle, although evidence for changes in ionic permeability and ionic fluxes during activity is less direct, it is probable that the upstroke of the action potential similarly results from a change in membrane permeability and inward sodium current.^{19, 30} It has been demonstrated that, during the upstroke of the action potential, the membrane resistance of Purkinje fibers is decreased to approximately 1/100 the resting value.²⁸ Furthermore, a decrease in the concentration of extracellular Na results in a marked drop in the rising velocity and magnitude of the action potential but fails to influence the resting potential.^{15, 31} Also in agreement with this mechanism is the similarity between the magnitude of the reversed membrane potential at the peak of the action potential (inside positive with respect to outside) and the potential difference which might be expected from a Na activity ratio of 1:10 across a membrane exclusively permeable to this ion.

The mechanism responsible for repolarization of the cardiac fiber membrane is poorly understood. Recent evidence suggests that the descending limb of the initial spiked reversal is a result of a decrease in Na permeability.³² However, although it has been demonstrated that activity in cardiac muscle is associated with an increased net loss of K and that most of this loss occurs during or shortly after the action potential³³ there is no direct evidence for an increase in K permeability during repolarization. Measurements of membrane resistance suggest that ionic conductance is decreased below normal values all during the plateau and that there is no appreciable increase in conductance during the repolarization limb of the action potential.^{34, 35} Also difficult to evaluate are the results of recent experiments which show that a sudden increase in the concentration of K outside the fiber results in a shortening of the plateau and accelerated repolarization.³⁶

In summary, the following hypothesis might be proposed to explain the potential differences recorded across the membrane of cardiac muscle fibers. The resting potential depends most directly on the potassium concentration gradient and appears to result from the greater tendency of this ion to diffuse outward across the membrane. The contribution of sodium to this potential is small because of the relative impermeability of the resting membrane to Na. With depolarization there is a large and specific increase in permeability to Na and the resulting inward sodium current, driven by both a concentration and potential gradient, is responsible for the rapid upstroke of the action potential and the reversal of polarity or overshoot. The descending limb of the reversal results from a decrease in Na permeability to resting values. During the plateau the membrane is only sparingly permeable and no large change in potential is recorded. Repolarization may be the result of an outward K current but additional evidence in support of this process is needed.

Transmembrane potentials and excitability. A study of the relationships between the transmembrane potentials of single cardiac fibers and the excitability of the heart to applied stimuli is an interesting extension of the material presented in the preceding paper. A series of investigations of a similar nature, based on the recording of the monophasic (injury) potential has been summarized by Schutz.¹⁹

(a) *Threshold.* The concept of "threshold" can be understood best by a consideration of the time-course of the transmembrane potential following a series of stimuli which increase in intensity from subthreshold to threshold strength and finally elicit an action potential. In an experiment of this type (fig. 3) rectangular pulses of cathodal current (current passing outward through the membrane) are applied across the membrane of a single fiber. The weakest current gives rise to a small depolarization that lasts as long as the stimulus; after the cessation of current flow the membrane potential returns to resting values with an exponential time-course. As the stimulus is increased in intensity, equal increments of current give rise to progressively larger and larger depolarizations until, when the membrane potential falls to a certain level, a very rapid depolarization (the upstroke of the action potential) supervenes. These observations show that the "threshold" of the fiber is really a critical level of membrane potential (the threshold potential) at

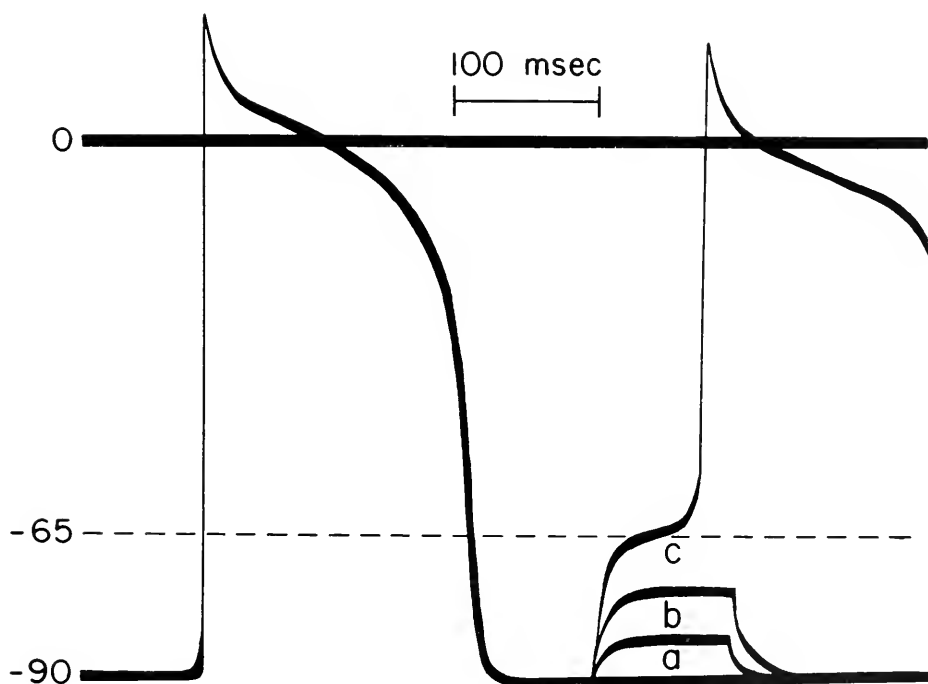


FIG. 3.—Subthreshold and threshold responses. Tracing of transmembrane action potentials of ventricular muscle (solid line) showing progressively larger depolarizations (a, b) resulting from two subthreshold stimuli and the production of an additional action potential by a threshold depolarization (c). Dotted line represents critical level of membrane potential (the threshold potential) at which self-sustaining depolarization eventuates. Time and voltage calibrations shown in figure.

which the increase in permeability of the membrane to Na is great enough to permit a self-sustaining depolarization. In other words, depolarization progressively increases Na permeability and at a critical level of membrane potential the net membrane current is inward and positive. Under this condition a rapid depolarization and reversal of the membrane potential results. This relationship between membrane potential and Na current has been studied in detail in the squid giant axon;³⁷ studies of isolated Purkinje fibers suggest that in the case of heart muscle the relationship is essentially as outlined.³²

The major factors which influence the strength of stimulus required to elicit a propagated response in cardiac muscle can be summarized quite briefly. As shown in figure 4, an effective stimulus must lower the transmembrane potential from the normal resting value to the critical or threshold level in order to elicit a propagated action potential. The stimulus requirement will thus depend upon both the absolute value of the resting potential as well as the value of the critical threshold potential. An additional factor of importance, especially when the stimulus duration is long, is the value of membrane resistance.

(b) *Pacemaker activity.* It is perhaps pertinent to discuss the nature of spon-

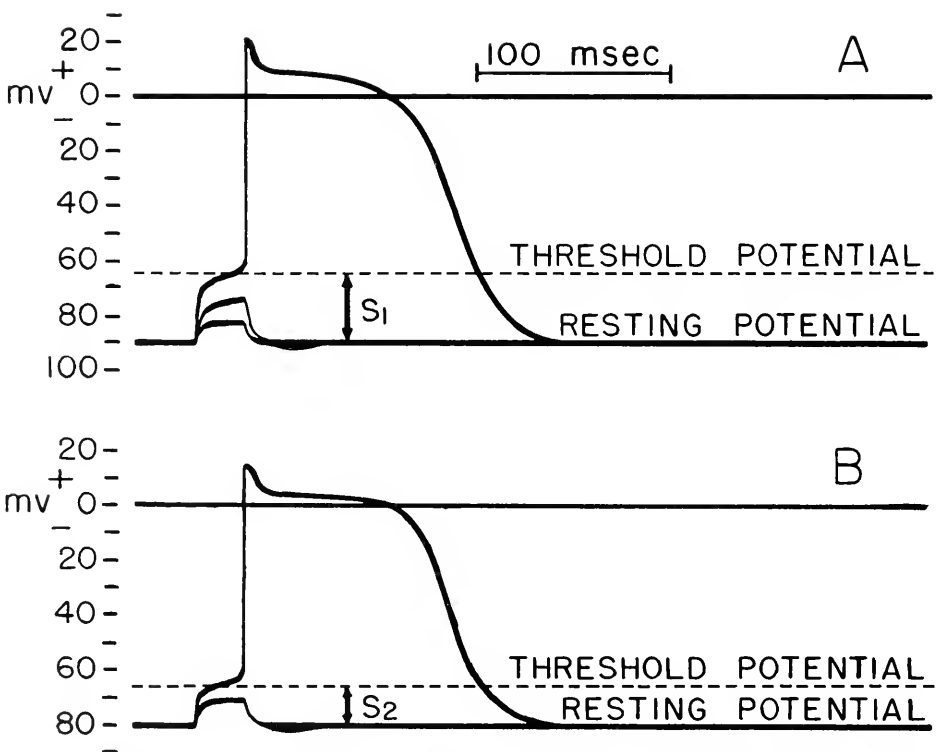


FIG. 4.—Diagrammatic representation of effect of changes in resting potential on the stimulus intensity required to elicit an action potential. Compare with figure 3. In A, a larger stimulus (S_1) is required to depolarize from resting potential (90 mv) to threshold potential (65 mv) than in B, where smaller stimulus (S_2) depolarizes from resting potential of 80 mv to threshold potential of 65 mv. Time and voltage calibrations shown in figure.

taneous rhythmicity in cardiac muscle at this time. Early studies of pacemaker activity in cardiac muscle suggested that spontaneous activity was associated with slow changes in membrane potential.^{38, 39} A direct demonstration of the membrane potential changes in a pacemaker was provided by microelectrode studies of isolated Purkinje fibers.¹⁵ This work revealed a consistent difference between records obtained from pacemakers and from other areas of the membrane. Records of the transmembrane potential obtained from a pacemaker showed that the resting potential begins to decrease immediately after the end of repolarization (fig. 5). When this slow diastolic depolarization reaches the critical level of membrane potential (the threshold potential) a rapid depolarization and formation of an action potential result. Typical records obtained from Purkinje fiber pacemakers show, in addition

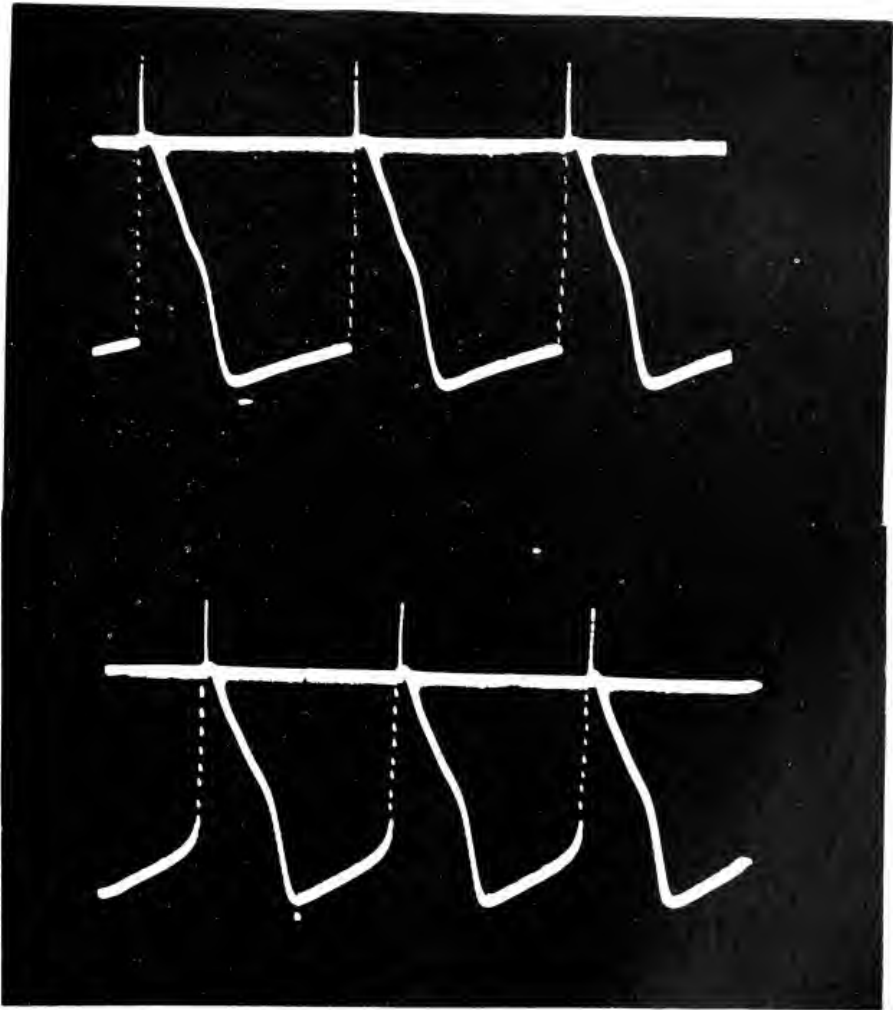


FIG. 5.—Transmembrane action potentials recorded from a single Purkinje fiber. Top: Records from potential pacemaker, showing slow diastolic depolarization. Bottom: Pacemaker records showing gradual transition from slow diastolic depolarization into action-potential upstroke.

to the slow diastolic depolarization, a slow upward curvature of the trace preceding the upstroke of the action potential and a slight decrease in magnitude of the reversal.^{19, 40} In adjacent areas of the membrane, driven by the primary pacemaker, the slow diastolic depolarization abruptly changes into the action potential upstroke as propagated activity arrives at the recording site (fig. 5). Records obtained from normal pacemakers in the S-A node⁴¹ and the sinus venosus^{42, 43} and from ectopic auricular pacemakers¹⁹ all show the same time-course of potential change initiating spontaneous activity.

(c) *Refractoriness.* The discussion of the refractory periods of the mammalian heart in the preceding paper have indicated that the recovery of excitability in cardiac muscle is a complex process, perhaps even more so than in the case of nerve or skeletal muscle. Although little is known of the basic mechanisms responsible for refractoriness, the changes in excitability encountered during repolarization can be partly explained by a consideration of the relationship between membrane potential and the change in Na current which results from a given degree of depolarization. Weidmann³² has studied this relationship in isolated Purkinje fibers by means of the "voltage-clamp" technique.³⁷ In this type of experiment the transmembrane potential of a given fiber is held at different steady levels of depolarization by means of current passed through one intracellular microelectrode and at each level of membrane potential the action potential elicited by a threshold stimulus is recorded by a second electrode in the same fiber. The maximum rate of rise of the action potential is employed as an indicator of the magnitude of the inward sodium current.

Results obtained by this type of investigation demonstrate that there is an S-shaped relationship between the steady level of membrane potential and maximum inward sodium current. Thus, while at resting potentials greater than 90 mv the rate of rise of the action potential is maximal, at a membrane potential of 70 mv it has fallen to one half and at a resting potential of 50 mv it is less than ten per cent of maximum. This relationship is seemingly responsible for the inability of the fiber to respond to maximal stimuli during the absolute refractory period, since if there is no inward sodium current there can be no action potential. Furthermore, the same property of the membrane can explain in part the elevated thresholds encountered during the relative refractory period. During repolarization, when the transmembrane potential is low, a stimulus of normal intensity results in only a small depolarization; this depolarization, in turn, causes only a negligible change in Na permeability and an action potential fails to occur. Stimuli much stronger than threshold, on the other hand, result in a large depolarization and the maximum Na current of which the membrane is capable at that instant. When this current density is adequate, a propagated action potential is formed. As repolarization proceeds toward completion, progressively smaller depolarizations result in propagated action potentials. It is also likely that part of the requirement for increased stimulus intensity during the relative refractory period is caused by the net outward current (possibly carried by K) which is responsible for repolarization of the membrane. This current opposes the depolarizing effect of the stimulus and raises the requirement for stimulus current.

The temporal relationship between the transmembrane action potential of ventricular muscle and the excitability of the membrane to applied stimuli can be sum-

marized as follows. The absolute refractory period begins with the upstroke of the action potential and lasts until repolarization is approximately two-thirds completed. The relative refractory period, in turn, extends from this point until shortly after the end of repolarization (fig. 6). Action potentials elicited by suprathreshold stimuli applied during the relative refractory period are altered in shape (fig. 7)

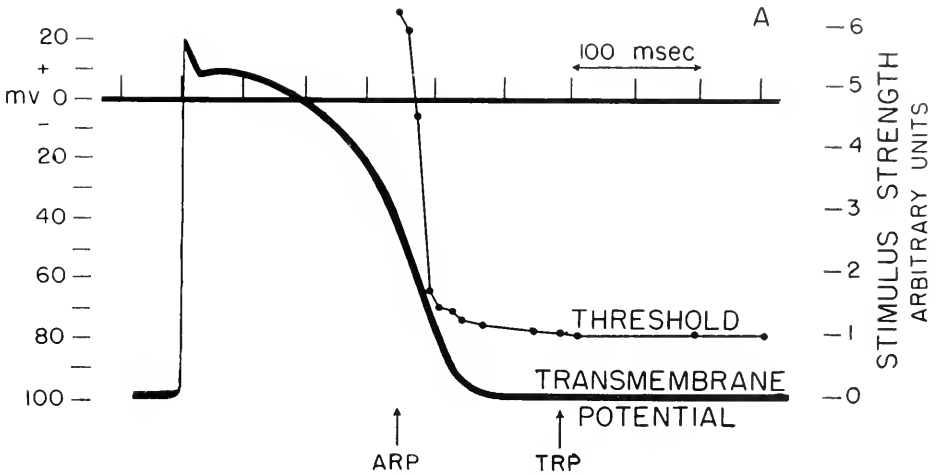


FIG. 6.—Tracing of transmembrane action potential of isolated ventricular fiber and curve of recovery of excitability. ARP = Terminal boundary of absolute refractory period; TRP = Terminal boundary of total refractory period. Time and voltage calibrations shown in figure.

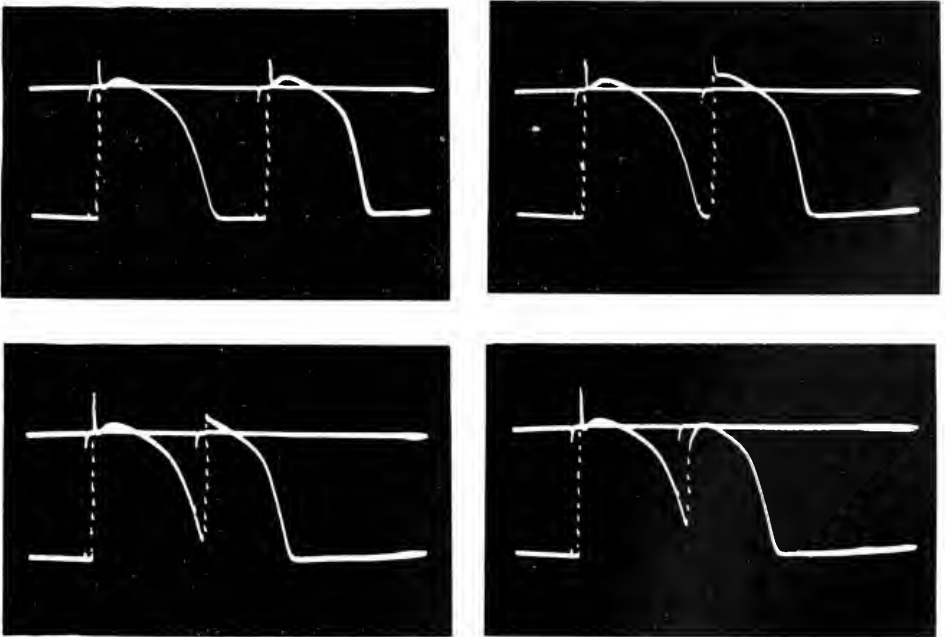


FIG. 7.—Action potentials recorded from a single isolated ventricular fiber showing the effect of prematurity on induced extrasystoles. Stimulus artefacts indicated by downward marks on baseline. Action potential upstrokes retouched. Note change in amplitude and duration of extrasystole.

and show a decreased rate of rise, diminished reversal, and lowered conduction velocity. In addition, the duration of the premature action potential is decreased and the plateau is less prominent.

No irregularity in the repolarization limb of the action potential is recorded which might be associated with the dips found in the curve depicting the recovery of excitability of the intact ventricle and auricle. On the other hand, studies of single isolated Purkinje fibers have revealed a definite period of supernormality just prior to the end of repolarization.⁴¹

When the membrane potential is only slightly higher than the critical threshold value, the stimulus current required to elicit a response is minimal (fig. 8). Subsequently, as repolarization progresses, the current required to produce a threshold depolarization is increased. This phenomenon is easily explained in terms of the relationship between critical threshold potential and resting potential (fig. 4) described above, and suggests that in this particular tissue recovery of excitability actually precedes recovery of normal polarization. The converse situation—com-

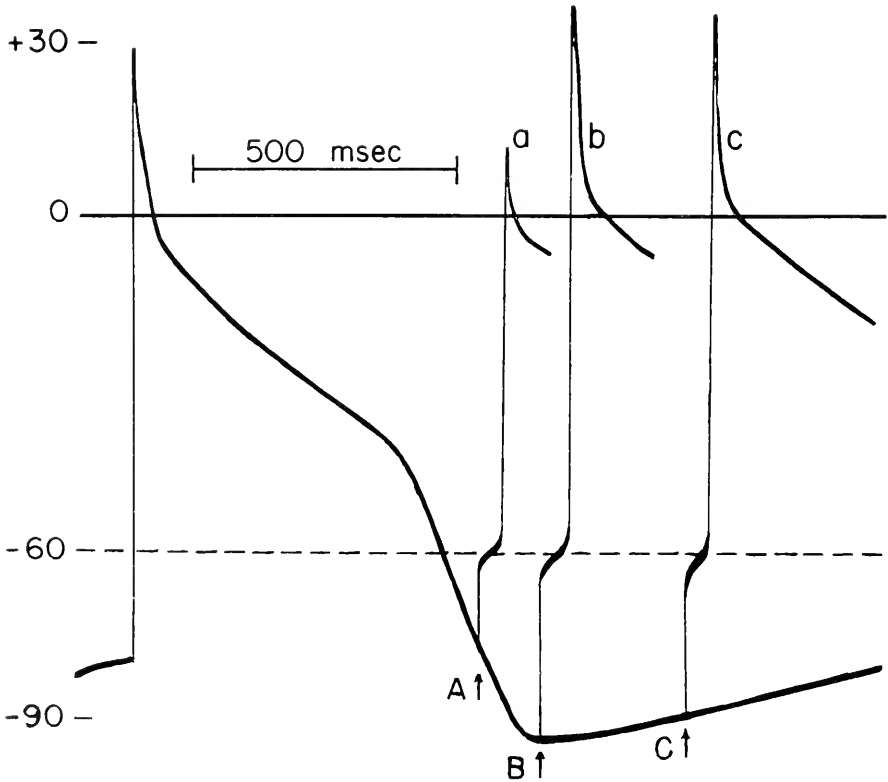


FIG. 8.—Tracing of transmembrane potentials of an isolated Purkinje fiber showing the occurrence of supernormality. Compare with figures 3 and 4. At A, a small depolarization resulting from stimulus (arrow) lowers the membrane potential to the critical threshold level and an action potential (a) ensues. After the end of repolarization (B) or during slow diastolic depolarization (C) stimuli stronger than A are required to depolarize the membrane to the critical threshold level at which firing of action potentials b and c occurs.

pletion of repolarization prior to recovery of excitability—will be mentioned subsequently in conjunction with the effects of low temperature.

The effects of temperature. The effect of temperature changes on the transmembrane potentials of single cardiac muscles have been studied extensively in recent years: frog ventricle;¹⁵ mammalian auricle;²⁹ mammalian Purkinje fibers;^{40, 46} mammalian papillary muscle.^{19, 47} An attempt will be made to summarize the results of these studies and to relate changes in membrane activity of the single cell to the phenomena associated with cooling of the intact heart *in situ*. In certain instances reference will be made to studies of single nerve and skeletal muscle fibers; in these tissues certain fields of investigation have progressed beyond the scope of present knowledge of cardiac muscle.

(a) *The magnitude of the resting and action potential.* Cooling of an isolated Purkinje fiber preparation results in a small decrease in the magnitude of the resting transmembrane potential (-5 mv) and a small but consistent increase in the reversal of polarity at the peak of the action potential ($+8$ mv).⁴⁰ In the case of the isolated cat auricle²⁹ the change in action potential amplitude during cooling is quite similar. In this tissue the maximum change ($+10$ mv) is recorded between $24-28^{\circ}$ C. and is associated with a slight increase ($+2-3$ mv) in the resting potential. In the isolated papillary muscle of both cat⁴⁷ and dog¹⁹ a decrease in temperature results in a maximum action potential amplitude at approximately 25° C.

If the temperature is lowered below 25° C. the changes in the action potential and resting potential are much greater in magnitude. Moreover, below this temperature the magnitude action potential and reversal decreases rapidly. In Purkinje fibers⁴⁰ the resting potential falls to low levels ($50-60$ mv) in the temperature range of $20-10^{\circ}$ C. and simultaneously the action potential is greatly diminished in height. Similar results are obtained from the isolated papillary muscle.^{19, 47} In both tissues the action potential often fails to show any reversal at the lower temperatures. The cat auricle fails to conduct at temperatures below 23° C.²⁹ Information concerning the action potential at lower temperatures is not available.

The changes in both resting potential and action potential occur almost instantaneously during cooling and are completely reversed with equal speed by rewarming. This observation suggests that the changes in magnitude of the resting potential and action potential induced by cooling do not result from changes in the concentration gradients of ions across the fiber membrane. Attempts to explain the observed changes in terms of other mechanisms, however, are uncertain. The increase in magnitude of the reversal, at the peak of the action potential, probably results quite simply from the relative difference in the effect of cooling on factors causing depolarization and repolarization. The rising phase is relatively insensitive to cooling, while repolarization is markedly slowed (see below); the action potential thus tends to approach an upper limiting value dependent upon the Na concentration gradient. Slight changes in resting potential can be explained on the basis of the effect of temperature on an ion concentration potential.⁴⁸ The marked decrease in resting potential at lower temperatures is more difficult to account for. It has been demonstrated that cooling decreases the activity of the sodium-pump and as a result the rate of active transport of Na outward and K inward across the membrane is diminished.¹¹ The decrease in resting potential thus might result from either an

accumulation of K in the extracellular space, or a relatively greater decrease in the outward Na transport than in inward K transport. Either of these effects would decrease the resting potential. The marked decrease in the amplitude of the action potential at low temperatures (10–20° C.) certainly results in part from a direct effect of the decreased resting potential on the amplitude of the action potential.³²

(b) *Rate and rhythmicity.* Records obtained from the pacemaker of a single isolated Purkinje fiber during changes in temperature clearly reveal the nature of the rate changes associated with heating and cooling.⁴⁰ The records reproduced in figure 9 show that, as temperature is lowered from 38 to 25° C., the slope of the slow diastolic depolarization of the pacemaker is decreased. Because this loss of resting potential proceeds at a decreased velocity the critical threshold level of membrane potential is attained only after longer intervals of time and frequency of action potentials is diminished. It should be noted, however, that the absolute value of the membrane potential at which self sustaining activity occurs (the critical threshold potential) is not altered; the threshold of the fiber, therefore, is not changed between temperatures of 38–25° C. Rate changes thus result from temperature effects on slow diastolic depolarization rather than from alterations in excitability.

At this time little is known about the mechanism responsible for slow diastolic depolarization of pacemaker tissue. It has been observed that a decrease in the concentration of extracellular sodium slows the rate of spontaneous activity⁴⁰ and that this slowing is associated with a decrease in the slope of diastolic depolarization.¹⁵ It is possible, therefore, that the depolarization characteristic of pacemakers results from a high sodium permeability of the membrane and greater than normal inward Na current during diastole. On the other hand, the high temperature coefficient of the slope of diastolic depolarization— $Q_{10}=6.2$ between 40–25° C.⁴⁰—sug-

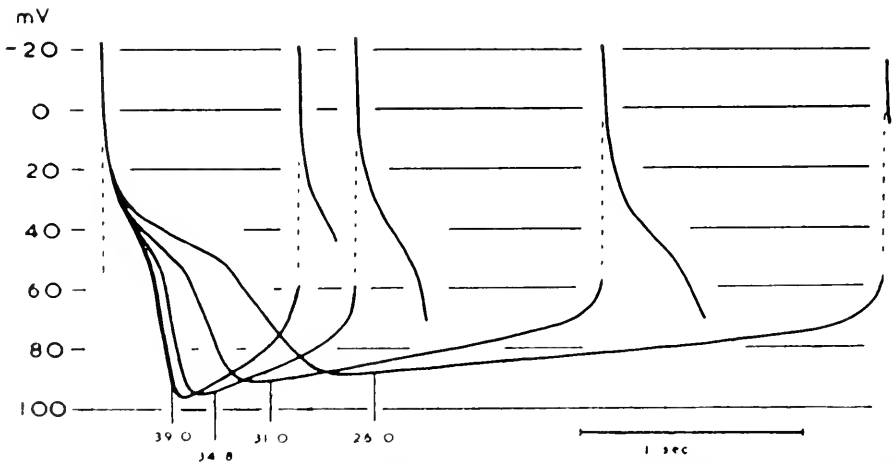


FIG. 9.—The effect of temperature on pacemaker activity in a single Purkinje fiber. Temperature in degrees centigrade for each action potential indicated in figure. Note that cooling decreases the rate of slow diastolic depolarization but does not alter the level at which the action potential upstroke ensues. Also note changes in action potential duration. Adapted from Corabocuf and Weidmann (40).

gests that the change in rate during cooling does not result solely from the effect of temperature on the diffusion of ions but that more complex reactions are concerned with the membrane properties peculiar to pacemaker regions of cardiac fibers.

Spontaneous activity ceases in isolated Purkinje fibers at temperatures between 25–15° C.¹⁰ in most instances, although occasional beats do occur at temperatures as low as 10° C. In addition, if the progressive change in membrane potential of a single fiber is studied during cooling, it is found most frequently that activity ceases during diastole, the resting potential remaining at a low level (50–60 mv). In certain cases, however, activity ceases *during* an action potential and the transmembrane potential of the fiber stays at the level of the plateau (near zero). In this case repolarization of the membrane does not begin until the fiber has been rewarmed.¹⁰

(c) *Refractoriness.* Changes in the duration of refractoriness resulting from a decrease in heart temperature have been discussed in the preceding paper. In auricle, ventricle, and specialized conducting tissues low temperature results in an increase in duration of both the absolute and relative refractory periods.⁵⁰ Studies of the transmembrane action potentials of these three tissues during cooling reveal changes in the time-course of repolarization mainly responsible for these alterations in refractoriness. In the case of the Purkinje fiber, the duration of both the plateau and the final phase of repolarization are increased by low temperature.^{10, 46} The relative change in the plateau, however, is considerably greater than in the final phase of repolarization. During rapid cooling of the isolated papillary muscle the alterations in these two subdivisions of the action potential are similar in nature—the relative prolongation of the plateau predominates (fig. 10). From these results

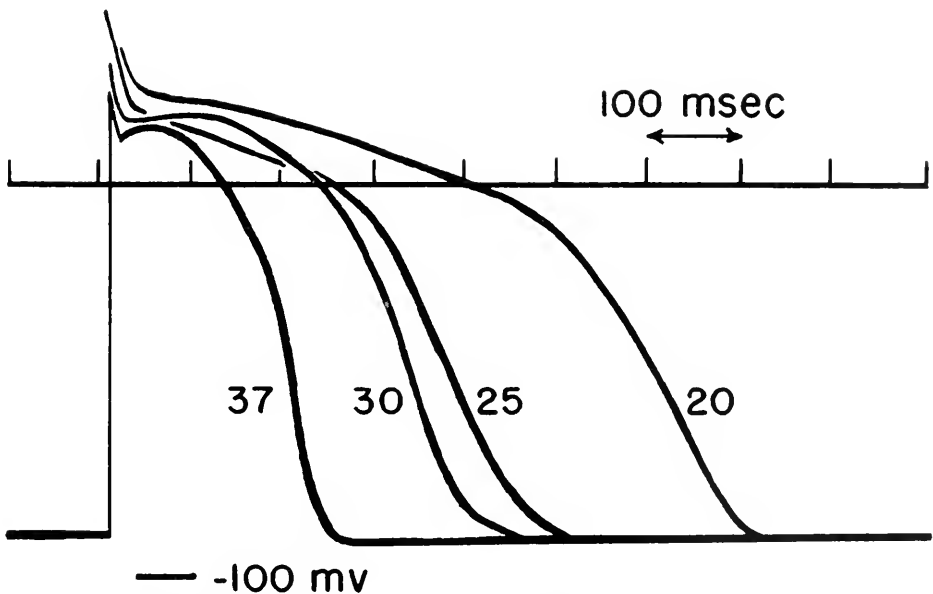


FIG. 10.—Tracings of transmembrane action potentials of a single isolated ventricular fiber in the dog papillary muscle showing the effect of rapid cooling. Temperature in degrees centigrade shown next to appropriate curves. Time and voltage calibration shown in figure.

it is apparent that absolute refractoriness should increase in duration much more than the phase of relative refractoriness when the heart temperature is lowered. In auricular muscle, since the action potential does not normally reveal a definite plateau, the change in repolarization consists mainly of a progressive increase in the duration of the final limb of the action potential. In some cases, however, cooling gives rise to a definite inflection in the auricular action potential which resembles the plateau of ventricular records.²⁹ Similarly, in some cases the time-course of repolarization of the papillary muscle is altered so that a definite plateau is no longer apparent.⁵¹

It is difficult to compare the degree of prolongation of the action potentials recorded from auricle, ventricle, and conducting tissues unless all preparations are driven at the same rate and alterations in frequency of contraction are prevented during the decrease in temperature. Comparison of available data^{19, 29, 40, 46, 47} indicates that the relative prolongation is similar in all three cases within a temperature range from 38–25° C. However, at lower temperatures there is often a marked increase in the action potential duration of certain preparations for a slight decrease in temperature and thus parallelism between different tissues is lost.

At 38° C. full excitability is restored at the same time as, or very shortly after, the completion of repolarization. This temporal coincidence of the recovery of membrane potential and excitability is not necessarily maintained at all times.^{10, 19} In the case of the isolated papillary muscle stimulated through surface electrodes, it has been shown that cooling delays the recovery of excitability considerably after the end of membrane repolarization. At temperatures between 13–14° C. (fig. 11) the duration of the absolute refractory period may exceed twice the duration of the action potential. It is certain that this change in duration of refractoriness is not a result of slowed conduction; the mechanisms responsible, however, remain uncertain.

(d) *Conduction velocity.* Low temperature is known to decrease conduction velocity in the auricle and ventricle of the mammalian heart⁵² and to slow propaga-

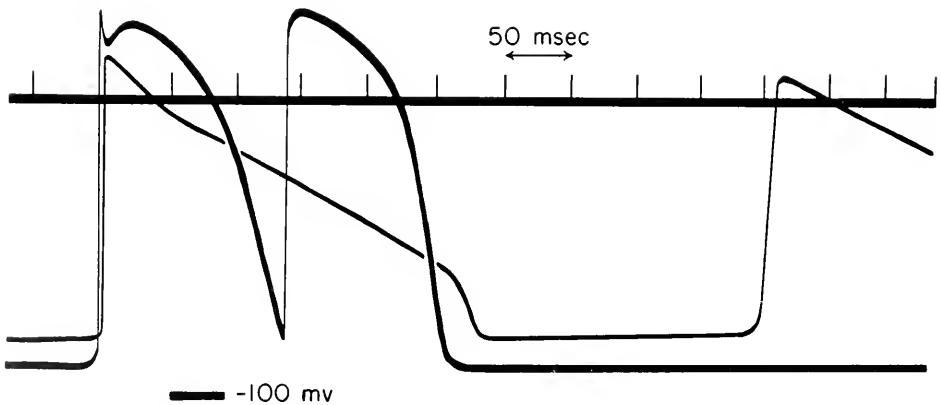


FIG. 11.—The effect of temperature on the recovery of excitability in ventricular muscle. At left, action potential and early extrasystole at 38° C. Shows decrease in amplitude and increase in duration. Earliest possible extrasystole at 13° C. shown at right. Time and voltage calibrations shown in figure.

tion of impulses across the specialized junction at the atrio-ventricular node.⁵³ The change in transmembrane action potentials most directly related to this decrease in conduction velocity has been studied recently in isolated Purkinje fibers.⁴⁹ In this tissue it has been shown that a decrease in temperature results in a slower rising velocity of the action potential ($Q_{10}=1.7$ between 40–25° C.). Similar results have been obtained from studies of nerve⁵⁴ and skeletal muscle.¹¹ This decrease in upstroke velocity results in slower conduction because, in any given area of the membrane, the critical level of membrane potential (threshold potential) is attained less rapidly. Similarly, a smaller area of membrane is depolarized in advance of the propagating action potential. At lower temperatures, when the resting potential is decreased, the diminished amplitude of the action potential will further decrease the stimulating efficacy of the upstroke and result in greater slowing of impulse propagation. In both auricular and ventricular muscle there is also a decrease in the rate of depolarization of the membrane at low temperatures; quantitative data permitting comparison with the Purkinje fibers, however, are not available.

The nature of the changes in A-V conduction resulting from cooling have not been directly studied by the microelectrode technique. However, the early occurrence of partial block may result from disproportionate changes in the duration of refractoriness in auricular and specialized fibers. Complete block, on the other hand, most likely reflects the inability of auricular muscle to conduct impulses at temperatures below 23° C.²⁹ This failure of conduction in auricular muscle contrasts with both ventricular and specialized fibers. In the former propagation is often maintained down to temperatures of 12–10°; in Purkinje fibers spontaneous propagated activity may persist until the temperature has reached similar low levels.

Effects of pH and P_{CO_2} . Heart muscle is reported to be relatively insensitive to changes in pH.^{55, 56} Studies of the transmembrane potentials of single ventricular fibers tend to support this statement. In this work the pH was varied by changing the concentration of bicarbonate buffer in Tyrode's solution aerated with a constant mixture of CO₂ and O₂.⁵¹ Between pH values of 6.5 to 8.0 the membrane activity of isolated ventricular muscle remains essentially unchanged. There are no significant alterations in the magnitude of the action potential or resting potential, and only minor changes in duration of the action potential. These changes in duration, decreased by low and increased by high pH, are similar to those produced by alterations in the concentration of extracellular Ca (see below).

The effects of changes in the partial pressure of CO₂ are more dramatic. In studies of isolated Purkinje fibers the P_{CO_2} was varied by changing the gas mixture employed to aerate a standard Tyrode's solution and transmembrane action potentials were recorded in the usual manner.⁵⁷ A gas mixture of 10 per cent CO₂ and 90 per cent O₂ resulted in marked slowing of spontaneous activity; the use of higher concentrations of CO₂ gave rise to repetitive firing, a decrease in amplitude of both resting and action potential and complete disorganization of activity. Unfortunately measurements of pH were not made in this study. However, in view of the minor effects of pH discussed above, it is likely that the changes noted are a result of the high P_{CO_2} . Although the partial pressure of CO₂ required to influence membrane activity in these experiments was quite high it is possibly not outside the

range present in the immediate environment of poorly perfused cardiac fibers during hypothermia.

Effects of potassium and calcium. The effect of increased extracellular potassium concentration on the resting transmembrane potential of single fibers is similar for nerve,⁵⁸ skeletal muscle,⁵⁹ and cardiac muscle.²⁹ The primary effect of high potassium is to decrease the magnitude of the resting potential²⁹ roughly in proportion to the change in K concentration. A direct result of the lowered resting potential is a decreased action potential amplitude and rising velocity (fig. 12). This effect, in turn, results in slower conduction. If the depolarization resulting from potassium is excessive, conduction fails and excitability is lost. These changes are similar in auricle, ventricle, and specialized conducting fibers although the Purkinje system appears to be most sensitive to high extracellular K.¹⁹ In all three

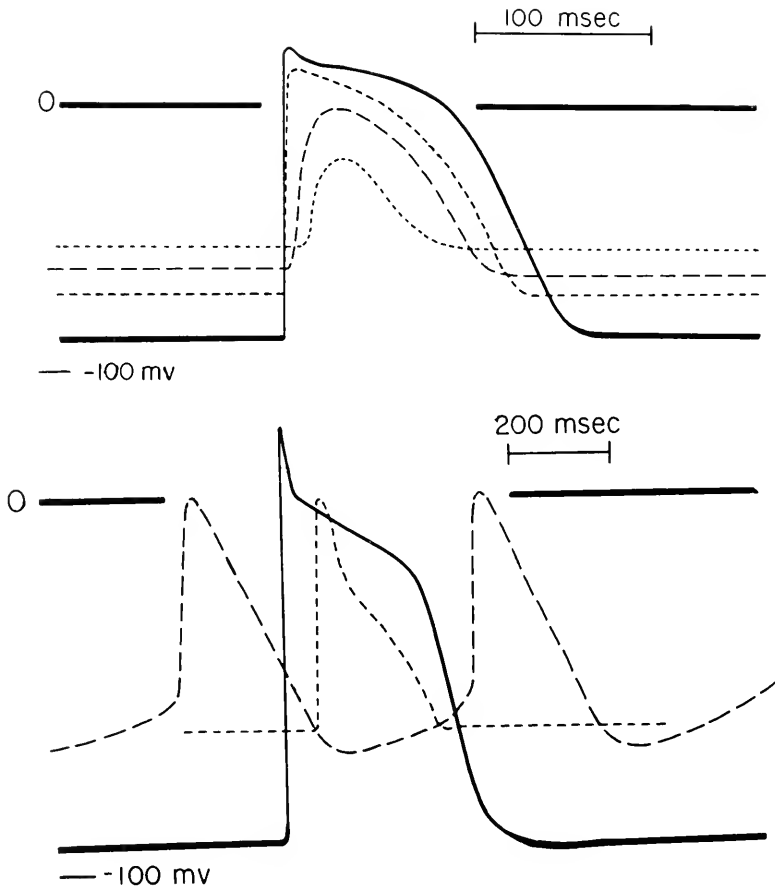


FIG. 12.—Top: Tracing of transmembrane action potentials of a single ventricular fiber showing control (solid line) and progressive decrease in resting and action potential produced by high extracellular potassium (dotted lines). Bottom: Tracing of control record from single Purkinje fiber (solid line) and effects of high (dotted line) and low (dashed line) extracellular potassium concentrations. Note similar changes in resting potential in both cases; increased pacemaker activity only with low potassium.

tissues the duration of the action potential is decreased by high potassium and the duration of the refractory period is shortened. The threshold of the membrane to external stimuli is first decreased and subsequently increased as the potassium concentration is progressively elevated.¹⁹

The nature of these changes in excitability and membrane activity can be explained in terms of the effect of K on the resting potential and the known relationships between resting potential, action potential and excitability (see above). If the resting potential of a fiber depolarized by high potassium is raised to the normal value by applied current, excitability is restored and action potentials showing the usual configuration and amplitude can be elicited.²² The change in action potential duration most likely also results from the low resting potential; however, a specific effect of high extracellular K on the rate of repolarization has been suggested by recent observations.²⁶

A decrease in the concentration of extracellular potassium also results in progressive depolarization of the fiber.¹⁹ In this case the changes in action potential configuration and amplitude are similar to those produced by K excess (fig. 12). Excitability and spontaneous rhythmicity, on the other hand, are greatly enhanced by low K until the resting potential falls below 55–50 mv.

The effect of potassium on the transmembrane resting potential is dependent upon the concentration of Ca in the extracellular fluid (fig. 13). High Ca protects the fiber from the depolarizing effect of elevated K and increases the depolarization resulting from a decreased K concentration. Low Ca has the opposite effect.¹⁹ This relationship is similar to that found in skeletal muscle.⁶¹ Changes in the calcium concentration have several other important actions on the transmembrane potentials.^{19, 44, 62, 63} Most important, perhaps, is the so-called stabilizing effect of ele-

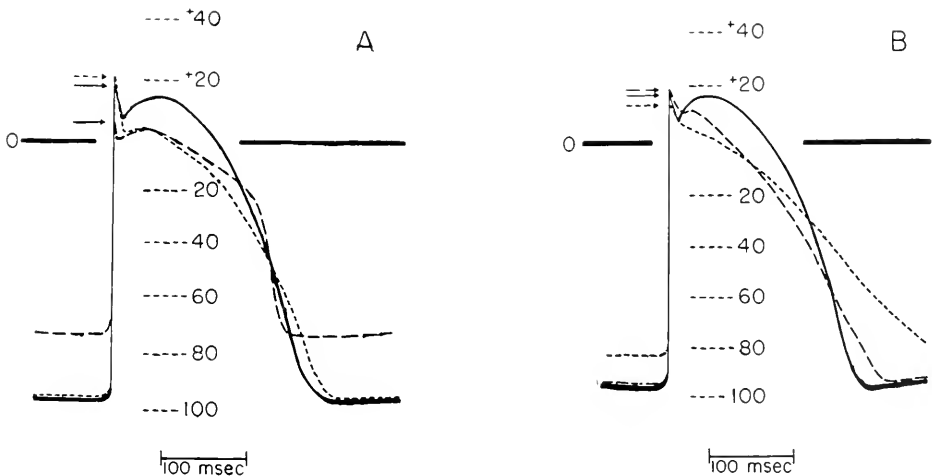


FIG. 13.—Tracings of transmembrane action potentials of single ventricular fibers showing effects of potassium and calcium. (A) Ca = $\frac{1}{10}$ normal, K = normal (solid line) = $\frac{1}{3}$ normal (dotted line) = 3 times normal (dashed line); (B) Ca = 3 times normal, K = normal (solid line) = $\frac{1}{3}$ normal (dotted line) = 3 times normal (dashed line.) Note that high Ca protects against high K, low Ca against low K. Arrows indicate peak reversal of appropriate action potentials. Time and voltage calibrations shown in figure.

vated calcium. In the case of the isolated Purkinje fiber elevated Ca increases the amount of depolarization required to stimulate and lowered Ca has the opposite effect. Decreases in rate in high Ca solutions are explained by this mechanism.⁴⁴ Although the configuration of the Purkinje fiber action potential is not altered by calcium concentrations between 0.7–10.8 mM, in the case of auricular and ventricular fibers marked changes in duration of repolarization are seen when the level of this ion in the extracellular fluid is altered (figs. 14 and 15).

Concluding remarks. It is apparent from the brief summary presented in the preceding pages that certain of the phenomena associated with cooling of the intact heart can be explained on the basis of information obtained from records of the transmembrane potentials of cardiac fibers. The decrease in heart rate which occurs during cooling results from a change in the slope of diastolic depolarization in pacemaker tissues. The occurrence of A-V block and idioventricular rhythms results from the great temperature sensitivity of auricular muscle, wherein conduction fails between 25–23° C., and the persistence of spontaneous activity in Purkinje fibers down to temperatures of 20–15° C. Changes in conduction velocity in the cooled heart are seen to result from the effect of temperature on the upstroke of the action potential; at temperatures below 25° C. conduction is also slowed by the low resting potential and resulting decrease in action potential amplitude. In addition, the lowered stimulating efficacy of these small and slowly rising action potentials may contribute to local conduction failure and disorganization of activity. The increased

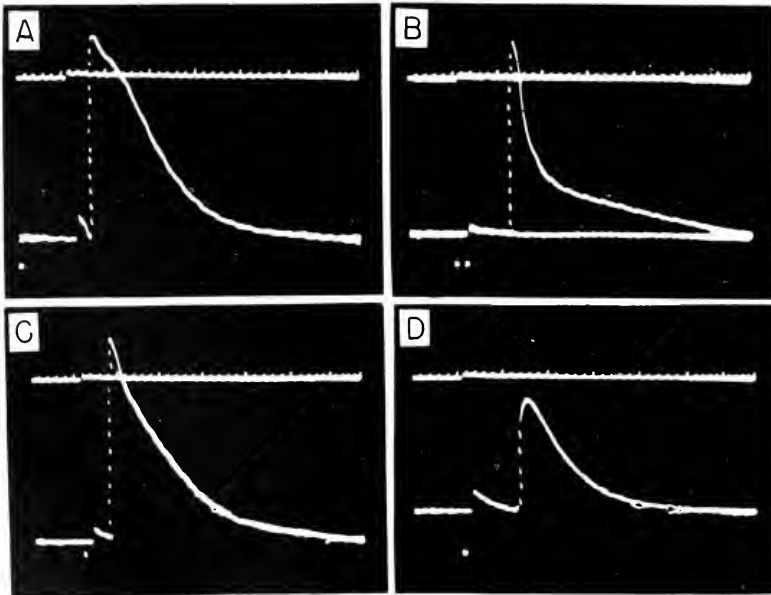


FIG. 14.—Effect of high and low calcium concentration on transmembrane potentials of auricular fibers. (A) Control, (B) 4 times normal (10.8 mM), (C) $\frac{1}{4}$ normal (0.65 mM), (D) $\frac{1}{10}$ normal (0.27 mM). At the high concentration (B) the fiber responds only to alternate stimuli. The response in (D) is not propagated. Time in 10 and 50 msec. Stimulus artefact before each potential.

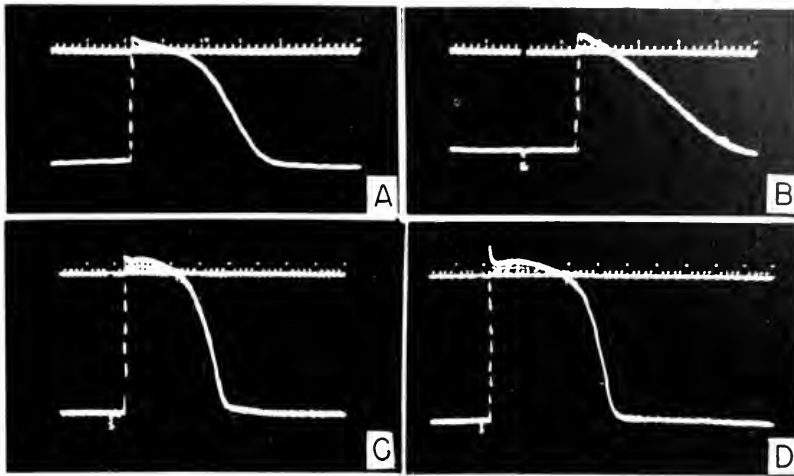


FIG. 15.—Effect of high and low calcium on transmembrane potentials of ventricular tissue. (A) Control record from a ventricular fiber. (B) The effect of a calcium concentration 4 times normal (10.8 mM). (C) Control record from a papillary fiber. (D) The effect of a calcium concentration of $\frac{1}{10}$ normal (0.27 mM).

duration of the relative and absolute refractory periods encountered during hypothermia is a direct result of changes in the duration of several components of the transmembrane action potential. The relatively greater prolongation of the plateau in comparison to the terminal phase of repolarization agrees with the observation that absolute refractoriness is prolonged relatively more than the relative refractory period by decreased heart temperature.

Excitability changes during hypothermia are varied. In some instances changes in threshold are not prominent⁵⁰ and this observation is supported by studies of the critical threshold potential of the isolated Purkinje fiber pacemaker. In other cases there is an abrupt loss of excitability in the intact heart. This occurrence may be due to the marked drop in resting potential seen in some fibers at temperatures below 25–20° C. and the known relationships between resting potential and excitability. On the other hand, loss of excitability may result from failure of repolarization which sometimes occurs during cooling.

In support of the statement that arrhythmias in the intact, cooled heart are not a direct effect of temperature on the myocardium is the observation that in isolated preparations of auricle, ventricle, and specialized conducting tissue, where changes in pH and P_{CO_2} are minimal, low temperature does not result in fibrillatory activity.

Studies of the transmembrane potentials of single cardiac fibers also support several other observations concerning the nature of excitability changes during hypothermia. Low temperature has been shown to result in a decrease in resting potential. Results obtained from studies of mammalian skeletal muscle¹² and isolated nerve fibers¹¹ as well as cardiac muscle demonstrate that this decrease in resting potential is associated with a loss of intracellular potassium. Several mechanisms might be responsible for this loss; important possibilities are: first, a direct effect of the membrane potential on K efflux, and second, a decrease in active

transport of Na or K. Regardless of the mechanisms concerned, potassium loss similar to that observed in intact hearts prior to fibrillation² is also encountered in the isolated preparation. Moreover, the decrease in intracellular K and increase in extracellular K may be associated with changes in a similar direction of the H ion concentration²⁴ and may explain in part the pH changes encountered in the intact animal. Finally, it has been demonstrated that changes in the concentration of extracellular Ca have a marked effect on the sensitivity of the membrane to changes in the level of K. This interrelationship may be of value in understanding the importance of the net changes in K and Ca fluxes across the membrane which have been associated with an increased likelihood of fibrillation in intact animals.²

In conclusion, certain major deficiencies exist in present day knowledge of the effects of temperature on the membrane and activity of cardiac fibers. Of primary importance are (a) information concerning the effects of additional variables (pH, K, Ca, P_{CO₂}) during cooling of cardiac muscle, and (b) studies of the metabolic activity and physico-chemical changes responsible for the phenomena observed.

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DISCUSSION

Dr. George E. Burch: I would like to ask two questions.

When one speaks of membrane permeability, is that strictly meant, or is it possible that there may be some alteration in molecular orientation or configuration which might produce polarization states, ionic states, for example, within the surface of the cell or some part of the cell?

Also, were sodium chloride, pH, and bicarbonate measured? A paper published in *Circulation Research* reported that in dogs changes attributed to potassium were also found to be produced by changes of sodium pH, bicarbonate or some other ion. Because of the complex relationship of some of these ions, one could get confusing impressions as to which ion was actually at work.

Dr. Hoffman: I can try to answer the second question very briefly: In terms of induced changes in extracellular cations, in all cases the simultaneous concentrations of sodium chloride, magnesium, etc. have been controlled. With very marked changes, let's say for example when potassium was increased by a multiple of ten, the total ionic strength of the Tyrode solution was maintained by decreasing sodium. In most instances we have maintained adequate control of the extracellular ionic concentration. In most of this work H ion and bicarbonate are reasonably constant. Even with changes in temperature we can keep pH well controlled.

With regard to the first question, as far as changes in membrane permeability are concerned, in speaking of the changes which occur during a normal action potential, the major evidence for a permeability change is probably the decrease and

then increase in membrane resistance simultaneous with the action potential. If one employs fluids containing only sodium or only potassium outside the fiber, one can get some information as to whether or not the increased conductance results from a change in sodium or potassium permeability. Furthermore, tracer studies have provided fairly good quantitative evidence of transmembrane ionic fluxes. However, I would not want to imply that other types of transport are excluded. I think that the metabolic extrusion of sodium against its electrochemical gradient and uptake of potassium are both largely dependent on energy-yielding reactions in the cell, and both are decreased very markedly by low temperature and by metabolic poisons. On the other hand the inrush of sodium during depolarization and the efflux of potassium during recovery seemed to be influenced only slightly by metabolic poisons, anoxia, or changes in temperature.

Dr. J. W. Severinghaus: I wanted to ask whether acetylcholine slowed the depolarization of pacemaker tissues the way one might expect.

Dr. Hoffman: As far as I know there are only two major effects of acetylcholine on these cardiac transmembrane potentials. In the case of the auricular muscle the rate of repolarization is greatly enhanced by acetylcholine. In the case of auricular pacemakers the slow diastolic depolarization is decreased. In addition, in certain cases when the resting potential is low, acetylcholine may restore it to normal values.

Dr. D. Durrer: In experiments on the excitability of the ventricular myocardium we have tried to investigate the individual roles which the anode and cathode are playing. When we investigate the cardiac excitability in the ordinary way we have the same results as Dr. Brooks has demonstrated.

However, we have a set-up which makes it possible to investigate the role of the anode and cathode separately. In an ordinary injection needle with a diameter of 0.9 mm., 10 or more small terminals are mounted so they are completely insulated from each other and from the shaft of the needle. Two of these small terminals are used as stimulating electrodes when the needle is applied to the ventricular myocardium. The terminals lying between the two electrodes are used for the detection of the point of origin of the extrasystolic beat. A bipolar lead is established by using two terminals close to the cathode electrode and another two are chosen close to the anode electrode. The first set tells what the cathode is doing, the second set what the anode is doing. Both bipolar complexes have the same polarity if the extrasystolic beat originates from cathode stimulation, and have the opposite polarity when the activation wave comes from the opposite direction, i.e. the anode.

With this method we believe we have proved that the strength/interval curve for stimuli delivered by the two electrodes in contact with the ventricular myocardium consists of two parts: an F part *before* the dip, which is of anodal origin, and a cathodal part after the dip.

In this way it was found that shortly after the absolute refractory period excitability response to anodal stimuli is maximal, and diminishes in the later part of the cycle. The excitability response to cathodal stimuli drops abruptly after the end of the absolute refractory period to attain gradually a constant diastolic level in 25–40 milliseconds.

The absolute refractory period (A.R.P.) for anodal stimulation of a region is

generally 2-15 milliseconds shorter than the A.R.P. of that same region for cathodal stimulation.

At the end of experiments designed for collecting other data on cardiac excitability we stop the respiration pump. From these "terminal experiments," we get the impression that if there is a greater time difference between A.R.P. for anodal and cathodal stimulation, the heart will fibrillate more readily during the period of anoxia which results from the stopping of artificial respiration. Experiments to make a more accurate analysis of this point are in progress. The results so far are conflicting.

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MYOCARDIAL IRRITABILITY IN EXPERIMENTAL IMMERSION HYPOTHERMIA

A. H. HEGNAUER AND B. G. COVINO

Hypothermia may be induced experimentally by a number of means and at various rates of cooling. Maintenance of the hypothermic state may be brief or prolonged, and at any level which is not immediately lethal. The condition has been studied in a wide variety of species either for comparative purposes or for specific measurements, each of which was more readily or economically accomplished in one than in another. The dog has been the principal species employed for the study of hypothermic myocardial irritability and the hypothermic state has generally been induced by immersion in cold water. This section is therefore concerned primarily with immersion hypothermia in the dog.

Terminus in acute experimental hypothermia in anesthetized dogs is either (a) ventricular fibrillation between 26° and 19° C., or (b) asystole between 18° and 14° C. The proportion of deaths in each category is related to the nature of the anesthetic administered preliminary to experimentation. Under light ether or thio-pental, and in the absence of extensive pre-immersion surgery, ventricular fibrillation terminates about 20 to 30 per cent of the experiments, whereas under pentobarbital anesthesia the rate is 60 to 70 per cent.^{1, 2} Seconal, in amounts required to block shivering, resembles pentobarbital in this regard.³ No attempt has yet been made to determine the basis for these differences, most studies having been made under pentobarbital anesthesia.

Several approaches to the problem of hypothermic ventricular fibrillation have been explored, primarily to discover practical means for its control. From the strictly physiological point of view the direct measurement of ventricular thresholds as modified by hypothermia would appear to be a fruitful starting point, and such an approach has recently been made and reported in a series of papers by Covino and collaborators, including the writer. Measurements of diastolic thresholds⁴ as well as of points earlier in the cardiac cycle were made.^{5, 6, 7} In the latter papers are given thresholds for early systole in some hypothermic dogs which are of a startling nature, and appeared to form a basis for the frequently observed ventricular fibrillation. These data have just recently been shown to be artefacts resulting from the particular arrangement of the stimulating circuits, the nature of which is at the moment only incompletely clear. Suffice it to say that the character of change in the refractory period of the ventricle in hypothermia is not correctly portrayed in those papers, and in fact there may be little if any change.

Diastolic thresholds in relation to character of death in hypothermia. For these measurements⁴ two stimulating electrodes were attached to the heart, one to the auricle to serve as pacemaker and the other to the left ventricle. A Grass Model 3C square wave stimulator drove the heart via the auricular electrode. The rate was maintained just sufficiently above spontaneous to assure control, and was appropriately reduced as body temperature decreased. The testing electrode on the ventricle led from a second stimulator which in turn was triggered by the first.

A variable delay circuit in the stimulator made possible the application of test stimuli at any point in the ventricular cycle, but such stimuli were delivered only when a manually operated switch on the testing stimulator was closed. The test stimuli were of constant 15 m. sec. duration and of variable voltage from 0 to 150. A 50,000 ohm resistor in series with the test electrode minimized the importance of possible tissue resistance change in the course of an experiment for purposes of computing current strength. Maximum stimulus intensities were thereby reduced to 2.73 ma. Diastolic excitability was measured at that point in the cycle represented by the descending limb of the electrocardiographic T-wave. Threshold was taken as the least intensity required to elicit a ventricular ectopic response.

A. *Controls (normothermia)*. To cool a dog to terminus by immersion in iced water requires 2 to 3 hours. Thus both time and temperature may be factors affecting ventricular excitability. To test whether fluctuations in diastolic thresholds might occur independently of temperature change, seven dogs under pentobarbital anesthesia were maintained at near normal temperature for 2.6 hours during which

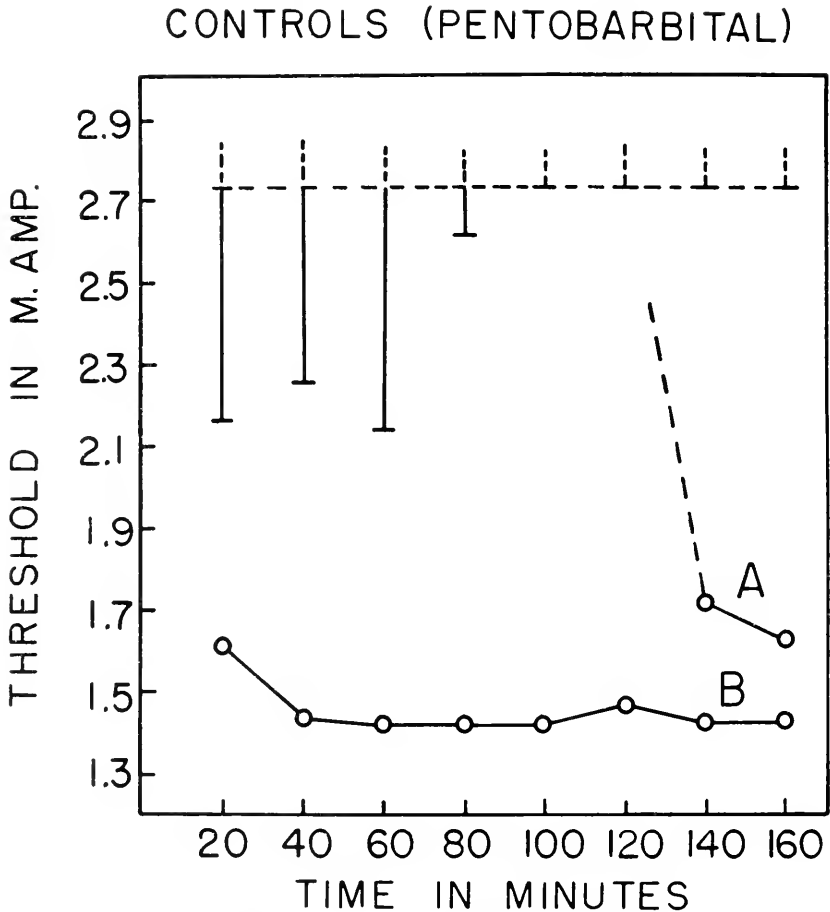


FIG. 1.—Ventricular thresholds vs. time under pentobarbital anesthesia. Solid vertical lines represent measurable range. Dashed vertical lines are thresholds beyond intensity range of apparatus employed. Curves A and B described in text.

time diastolic thresholds were measured at 20 minute intervals. The results are given in figure 1. Immediately after chest closure and resumption of spontaneous respiration thresholds are found to vary over a considerable range (2.1 to >2.73 ma.) in six of the dogs. After 1.25 hours, however, the thresholds of all have risen to a value greater than 2.73 ma. and (with one exception) remain there. The exception (curve A, fig. 1) remains unexplained. The data for curve B are from a dog which possessed ventricular arrhythmias prior to, as well as after, thoracotomy, and may therefore legitimately be ruled out as a normal control animal. A similar series of control studies under thiopental anesthesia yielded practically identical results. Thus after 1.0 hour all thresholds were greater than 2.73 ma. and remained so for the duration of the observations.

It appears, therefore, that time after completion of required surgery tends to raise the diastolic thresholds to higher values than obtained initially.

B. *Hypothermia*. Typical of the effects wrought by hypothermia are those represented by the data plotted in figure 2. The 15 dogs of this series were subjected to the same experimental procedures as those of the normothermic controls except

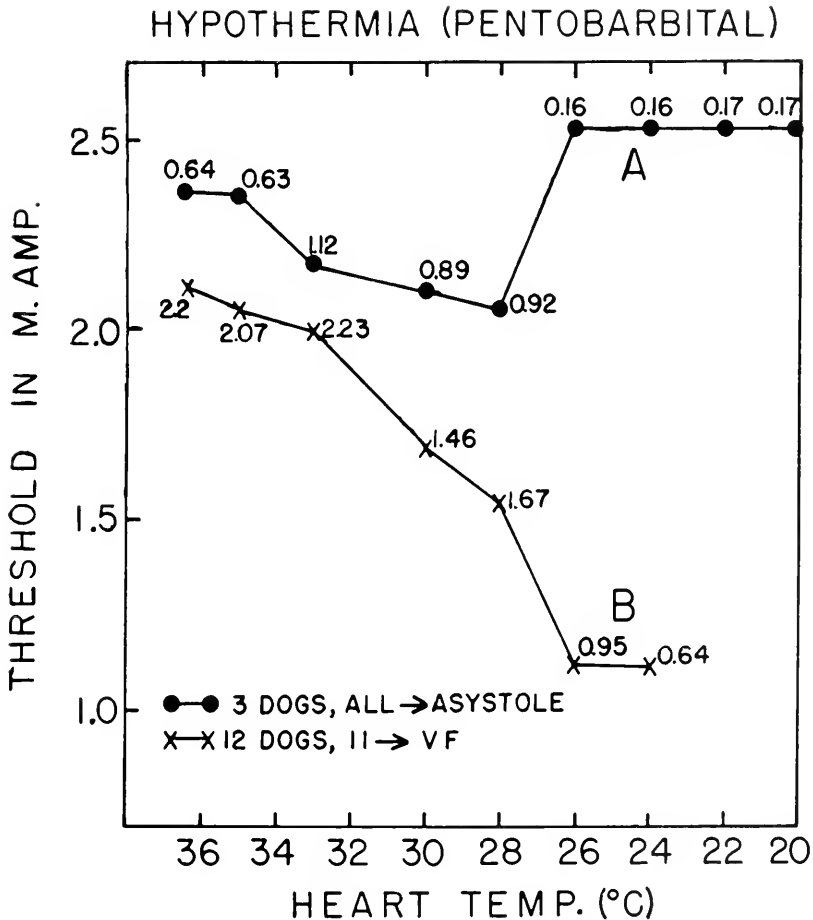


FIG. 2.—Ventricular thresholds (with standard deviations) of hypothermic dogs under pentobarbital anesthesia. See text for basis of separation into subgroups A and B.

for hypothermia induction. Analysis of the data after completion of the experiments revealed that a spontaneous separation into distinct subgroups had occurred, the distinction being evident only at low temperatures and at terminus. The apparently related phenomena upon which the separation is based are: (a) the degree of threshold decline with heart temperature, and (b) temperature and mode of death, i.e., ventricular fibrillation or asystole. The upper curve of figure 2 is a plot of the means of the thresholds of three dogs (subgroup A) all of which cooled to 16° to 18° before death in asystole. The numbers at each plotted point are standard deviations of the means, which become progressively smaller at the lower temperatures. Curve B is a similar plot for the 12 dogs of subgroup B, 11 of which succumbed to ventricular fibrillation between 20° and 26° C. Other interesting points in relation to these data are: (a) the difference between the two subgroups can be demonstrated statistically only after a temperature of 28° is reached, the difference being highly significant at 26° ($P < 0.01$), (b) there is no overlap of the ranges of the two subgroups at 26° or lower, (c) the range of thresholds of the group B dogs at 24° and lower is 0.27–1.4 ma. with a mean of 1.16, and although such low thresholds may (and did) obtain among both subgroups above 30°, fibrillation became associated therewith only after temperature had declined at least to 26°. It would appear from this and many other observations that low thresholds above 28° are not of prognostic importance in acute hypothermia, but become so at lower temperatures.

Similar measurements were made on a second series of 13 pentobarbitalized dogs in another connection (fig. 3) with practically identical results. And a third series of 15 experiments performed under thiopental anesthesia differed only quantitatively. In the latter series there were five dogs in subgroup A (high preterminal thresholds with asystolic deaths) and 10 in subgroup B. Ventricular fibrillation overtook seven of the latter between 23° and 19°. The other three survived to 15° to 18° without terminal fibrillation. This lower ratio of fibrillation to asystole under thiopental is in accord with previous observation, but an explanation for the better showing of thiopentalized animals awaits investigation.

In summary the data from 43 barbitalized dogs, respiring spontaneously to 25° during cooling, indicates that when a high diastolic threshold is maintained during hypothermia survival is assured to lowest temperatures and death will be asystolic (11 dogs). Failure to maintain or regain a near-normal threshold during hypothermia produces almost certain fibrillation above 19° (27 of 32). This is not to say that the intimate mechanism inducing fibrillation acts necessarily by way of the low diastolic threshold. The low diastolic threshold is probably an index of change elsewhere in the cycle of a nature which predisposes to fibrillation.

Ventricular excitability in relation to hypothermia and pH. The question may now properly be asked: Are the effects on threshold as observed in hypothermia due to temperature *per se*, or are they related only indirectly to temperature and more directly to other changes induced by low temperature? Since such factors as hypoxia, arterial blood pressure, central venous pressure, autonomic nervous activity, etc., had apparently been ruled out⁸ it became necessary to seek elsewhere for possible primary causes for the increased excitability and fibrillation. A report by Swan *et al.*⁹ that hyperventilation during the course of hypothermia

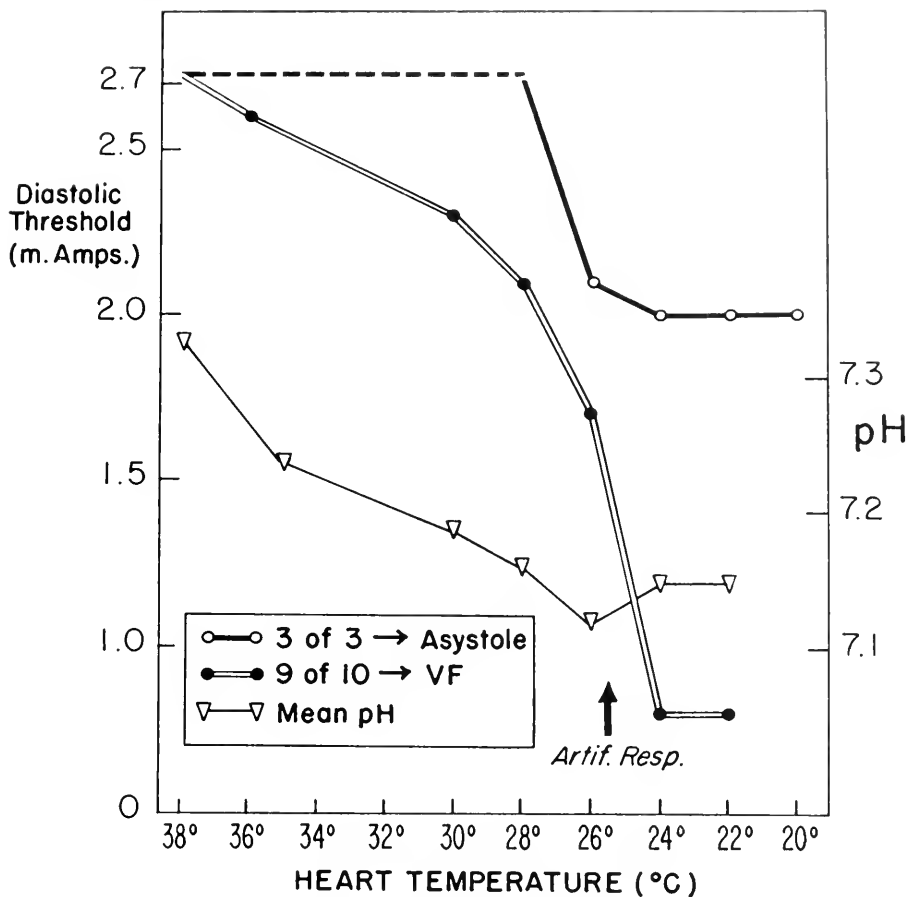


FIG. 3.—Diastolic threshold vs. heart temperature in hypothermic dogs when pH is permitted to drop below 7.2. Subgroups A and B as for figure 2. Dashed line is maximum stimulus intensity available.

decreased the incidence of ventricular fibrillation in dogs in which the circulation was temporarily arrested for experimental cardiac surgery, and the subsequent demonstration by Osborn¹⁰ that maintenance of a normal (or slightly supernormal) pH similarly decreased the number of fibrillary deaths, offered a promising lead. For if altered thresholds and fibrillation were intimately related then pH control should prevent the occurrence of both. The investigations based upon this hypothesis established that ventricular excitability too is greatly influenced by pH.⁶

In the experiments discussed to this point the dogs respired spontaneously to 24° to 26° and were artificially respired during further cooling. The artificial ventilation rate was made to approximate the immediately prior spontaneous rate and was not again varied. At 25° the mean systemic pH approximates 7.15, and this may rise slightly after artificial respiration is started. Prevention of a pH rise may be accomplished by substituting a 3 per cent CO₂-O₂ mixture for room air. In addition to pH stabilization the mixture with a high O₂ content assures adequate arterial

and (presumably) tissue oxygenation thus further eliminating hypoxia as a possible complication in the interpretation of the data. It is apparent from figure 3 that administration of the artificial gas mixture to the respired air did not materially affect the physiological status of the animals at least in respect to diastolic thresholds, lethal temperatures, and the distribution between asystolic and fibrillary deaths (compare with figure 2).

In contrast to the data of figures 2 and 3 are those obtained from dogs subjected to the same conditions except that throughout cooling they were respired artificially at a rate to maintain blood pH within the range 7.3–7.55. From figure 4 it may be noted that all diastolic thresholds remained above the apparently critical level of 1.4 ma., even at the lowest temperatures. Death was due to asystole in all cases, and at a mean temperature of $16.9^{\circ} \pm 1.6^{\circ} \text{C}$.

This evidence that maintenance of an approximately normal pH during the course of hypothermia induction will (a) diminish the frequency of ventricular fibrillation, and (b) hold ventricular excitability at a normal level, suggests that a common underlying mechanism is in some measure influenced by pH. It is highly probable that this mechanism is concerned with myocardial electrolyte and mineral balances (a subject to be discussed presently). Extreme alkalosis, too, may induce modifications in ventricular excitability which lead to fibrillation. Thus in two additional dogs the ventilation rate was inadvertently excessive during cooling, with a consequent elevation of pH to 7.8. Ventricular fibrillation terminated both experiments above 20° . This may be compared to the experience of Osborn¹⁰ who found that hypothermic dogs whose pH exceeded 7.6 during the early stages of cooling were extremely susceptible to fibrillation. Thus the optimal pH range for hypothermic dogs appears to be that of the normal, i.e., 7.3–7.55.

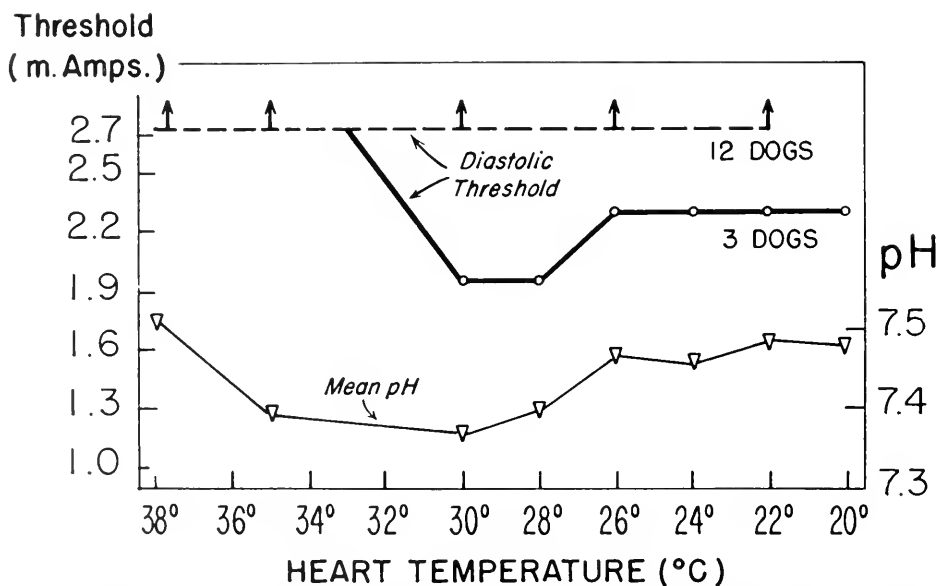


FIG. 4.—Diastolic threshold vs. heart temperature in hypothermic dogs when pH is maintained between 7.3 and 7.55. Thresholds above 2.73 ma. given by vertical arrows.

Once a critical set of conditions has been established by low pH and temperature it does not appear readily reversible by simple restoration of a normal pH. In a series of 10 acidotic hypothermic dogs (mean pH at 25°=7.16) a normal pH was re-established in 5 to 10 minutes by instituting artificial hyperventilation at the 25° level. During further cooling 7 of the 10 succumbed to fibrillation within 30 to 90 minutes after the start of augmented respiration, i.e., between 23° and 18°. The only beneficial effect of pH restoration thus appears to be a slight lowering of the temperature at which fibrillation occurs. Miller *et al.*¹¹ found that when acidosis was induced in normothermic anesthetized dogs by CO₂ inhalation, a sudden shift back to normal pH was usually followed by ventricular fibrillation within 2 to 10 minutes.

Hypothermia and pH on the ECG. Bigelow, Lindsey, and Greenwood¹² were the first to note a feature of the ECG which is common in simple hypothermia. When present it is typified by a wave rising steeply from the S-wave, producing an elevation of the early portion of the S-T segment. Osborn¹⁰ later showed that the S-T elevation (termed "injury current" by him) was present in all but one of the dogs in his experimental series which later succumbed to ventricular fibrillation, and only rarely in dogs which cooled to low temperatures and terminal asystole. Support for the view that it is a bad prognostic sign is an additional analysis of ECG records of 49 hypothermic dogs cooled to terminus.⁵ The "injury current" was present in 28 of the 36 fibrillators (78%) and in 6 of the 13 non-fibrillators. Examination of the few extant ECG records of human victims of accidental hypothermia reveals the presence of the abnormal wave in each.^{13, 14, 15} One of these¹⁵ survived a rectal temperature of 18° C.

The term "injury" current or potential is perhaps a misnomer when applied in hypothermia. In the first place it is not associated in these experiments with either gross or microscopic injury. It is readily reversible by rewarming, as seen in one published record¹⁶ and in many unpublished. According to Osborn¹⁰ it is reversible at low temperature by raising blood pH to a normal value. In the experiments described earlier in which pH reversal was carried out in 10 dogs at 25°, the seven fibrillators all had S-T segment elevations which, however, persisted to terminus. On the other hand, Altschule and Sulzbach¹⁷ made note of the presence of a similar wave in their unanesthetized human subjects breathing high concentrations of CO₂, and which disappeared on resumption of room-air breathing. It is not clear, therefore, whether the simple factor of temperature or the time that the wave is permitted to persist determines the ECG reversibility. And in all probability the same applies to the processes underlying excitability change and fibrillation as noted above.

Arterial and coronary venous electrolyte levels and differences. That alterations in blood electrolyte levels might in some manner be influential in determining the course of events in hypothermic animals has been considered by a number of investigators. Elliott and Crisman¹⁸ measured both potassium and calcium levels in the blood of anesthetized hypothermic rats, and noted a rise in concentration of both ions at low body temperatures. Bigelow¹² too found an increase in the serum potassium in hypothermic acidotic dogs. Fleming¹⁹ found no important deviations from normal with respect to potassium, calcium, sodium, or magnesium.

Swan *et al.*,⁹ and Osborn¹⁰ on the other hand found that the serum potassium level decreased in their hypothermic dogs.

The work of Osborn¹⁰ on pH and bicarbonate levels in relation to fibrillation and on ECG are of fundamental importance, and, together with the observation of Swan *et al.*,⁹ that hyperventilation reduces or abolishes spontaneous fibrillation, helped in directing closer attention to electrolyte balances.

Electrolyte levels and differences in the coronary circuit have been measured.²⁰ Three groups of hypothermic dogs were considered for mutual comparisons and for comparisons with their own status prior to hypothermia. These groupings, which have already been considered in relation to ventricular thresholds and the nature of terminal heart action are: (1) hypothermic, normocapneic dogs (none of which fibrillate spontaneously), (2) hypothermic, acidotic dogs which fibrillate terminally, and (3) acidotic hypothermic nonfibrillators. Dogs artificially respired during cooling for pH control were automatically in group 1, whereas the grouping of the acidotic (spontaneously respiring) hypothermic dogs was made on the basis of terminal cardiac action.

Coronary venous samples were taken from the great circumflex vein both at normal temperature and after cooling to 24°. After each sample the chest was closed and the pneumothorax reduced, permitting spontaneous respiration in groups 2 and 3, and cooling was continued to terminus.

The electrolytes measured in all samples were sodium, chloride, potassium, calcium, magnesium and hydrogen ions (pH). Summaries of the coronary A-V differences are given in tables I and II. Table I contains the means (and standard

TABLE I

CORONARY A-V ELECTROLYTE DIFFERENCES IN M $\bar{E}Q$. PER LITER IN TWENTY-SIX DOGS PRIOR TO SUBJECTION TO IMMERSION HYPOTHERMIA

	Total					
	pH	Ca	K	Na	Cl	Mg
Group 1	+0.01	-0.2	+0.10	-2.0	-3.0	-0.20
8 normocapneic non-fibrillators	(± 0.02)	(± 0.24)	(± 0.10)	(± 2.0)	(± 1.6)	(± 0.08)
Group 2	+0.02	-0.1	0.0	-5.0	-0.5	-0.20
10 hypercapneic fibrillators.....	(± 0.01)	(± 0.18)	(± 0.13)	(± 3.2)	(± 1.2)	(± 0.12)
Group 3	+0.03	-0.2	+0.12	-6.0	0.0	-0.20
8 hypercapneic non-fibrillators	(± 0.01)	(± 0.22)	(± 0.18)	(± 2.4)	(± 2.0)	(± 0.04)

TABLE II

CORONARY A-V ELECTROLYTE DIFFERENCES IN M $\bar{E}Q$. PER LITER, MEASURED IN THREE GROUPS OF HYPOTHERMIC DOGS AT 24° C.

	Total					
	pH	Ca	K	Na	Cl	Mg
Group 1	+0.03	-0.2	-0.1	-3.0	+2.0	-0.2
8 normocapneic non-fibrillators.....	(± 0.01)	(± 0.15)	(± 0.16)	(± 2.8)	(± 1.5)	(± 0.04)
Group 2	+0.08	+0.4	-0.2	-3.0	0.0	0.0
10 hypercapneic fibrillators	(± 0.04)	(± 0.34)	(± 0.59)	(± 2.6)	(± 0.97)	(± 0.11)
Group 3	+0.03	-0.1	0.0	-3.0	+1.5	-0.3
8 hypercapneic non-fibrillators.....	(± 0.01)	(± 0.16)	(± 0.18)	(± 3.2)	(± 1.8)	(± 0.07)

deviations) of the several coronary A-V electrolyte differences in all groups prior to immersion. The plus sign indicates higher arterial than coronary venous values, presumably signifying a positive balance with respect to the myocardium. Exceptions must be made for pH (which if expressed in terms of $[H^+]$ would be prefixed with a minus sign) and sodium, the latter being significantly higher in coronary venous than arterial blood at normal and low temperatures in all three groups. The magnitude of the sodium difference is such that it cannot reasonably be ascribed to a continuous negative cardiac balance, for the cardiac reserves are not greater than those of other tissues. But an alternative explanation for the difference is elusive.

Since neither the levels (not shown in the tables) nor coronary A-V differences for sodium, chloride and magnesium were demonstrably affected by hypothermia in any of the three groups these ions will not be further considered. The ions apparently affected in one or another group at low temperature were calcium, potassium and hydrogen. And these will now be discussed.

In all three groups the systemic and coronary venous plasma potassium levels were less at low than at normal temperature by a mean of 0.6 mEq. per liter, in confirmation of other observations.^{9,10} And since the magnitude and direction was unaffected by pH the decrease must for the present be assumed to be intimately linked with the temperature factor. With respect to coronary A-V differences and systemic blood levels of calcium, a temperature effect was not demonstrable. Left for consideration therefore are coronary A-V differences in hypothermia as influenced predominantly by systemic pH.

In the group-2 dogs (acidotic fibrillators) coronary A-V differences of potassium, calcium and hydrogen ions are present. Potassium and hydrogen ion concentrations are higher in the venous blood and calcium is lower. The potassium difference of 0.2 mEq. per liter is not statistically significant, but becomes so at the 5 per cent level if the data of one dog are excluded for analytical purposes. The one exception showed a *lower* venous than arterial potassium level of a magnitude beyond the range shown by any other dog in any of the three groups. The tremendous deviation in the opposite direction accounts also for the large standard deviation shown in the table.

The mean calcium A-V difference in the same group-2 dogs is 0.4 mEq. per liter and is statistically significant ($P = < 0.02$). Two dogs in this group failed to show lower total venous than arterial calcium levels. The nonfibrillators, whether acidotic or with normal pH values, presented either no difference or a slightly higher venous level.

The pH measurements as given in table II are also revealing. The pH of coronary venous blood at normal temperature for all groups, and at 25° for groups I and 3, averages 0.03 units less than arterial. In no individual instance was a difference as great as 0.05 units observed. Among the fibrillators (group 2) on the other hand are none with a coronary A-V pH difference as low as 0.05 units, and the mean for the group is 0.08. In fact it became apparent that the observer could predict the fate of each dog at 25° from the A-V pH difference, i.e., whether terminus would be fibrillary or asystolic.

With the exceptions noted the data suggest that certain acidotic hypothermic

dogs succumb to fibrillation because their ventricular thresholds are lowered, and the lowering is in some manner the consequence of calcium penetration into the myocardium in exchange for potassium and hydrogen ions. Those acidotic hypothermic dogs which can maintain a normal electrolyte balance across the myocardial membrane succumb ultimately to the effect of temperature on metabolic processes responsible for energy-mobilization and release, i.e., death is asystolic.

A summary to this point reveals that a proportion of acidotic hypothermic dogs succumb to ventricular fibrillation between 25° and 19° whereas asystole at lower temperatures accounts for the remaining acidotic dogs and all of those with normal pH. The characteristics which appear to distinguish the fibrillators from the others are: (a) lower diastolic thresholds, (b) positive calcium and negative potassium and hydrogen ion balances with respect to the myocardium, and (c) elevation of the early portion of the S-T segment of the electrocardiogram. Since none of these characteristics has thus far been noted in hypothermic dogs possessing normal pH the conclusion that the acidotic rather than hypothermic state is the more important causal agent seems justified. Not ruled out by the observations to date, however, is the possibility that, although pH appears to dominate, temperature itself may be an ally. This point will be discussed further.

Exogenous calcium and fibrillation. On the premise that hypothermic spontaneous ventricular fibrillation is the consequence of (or intimately related to) the myocardial penetration of calcium, and that the latter is controlled in considerable degree by the systemic pH, one might conclude that the hypothermic acidotic dogs would be more sensitive to an elevation of the plasma calcium level than would non-acidotic dogs. To test the hypothesis, normothermic and hypothermic dogs were infused with isotonic calcium chloride at the rate of 5 ml. per minute.²¹ The hypothermic dogs were again subdivided into acidotic and non-acidotic groups. In addition to volumes (or m.eq. of calcium) infused to terminus, plasma levels and pH were measured in a proportion of dogs in each group. These are given in table III, together with total number of animals and the character of the terminal cardiac action.

Results show that 65 per cent of normothermic dogs terminate in ventricular

TABLE III

CARDIAC SENSITIVITY TO EXOGENOUS CALCIUM IN NORMAL AND HYPOTHERMIC DOGS

Heart temp.	All experiments VF/total	Number of dogs	Ca admin. mEq./Kg.	Fibrillators		Plasma mEq./L. init.	Ca term.
				pH Init.	pH Term.		
38±1	11/17	5	{ 5.4 ±1.4 }	7.39	7.42	{ 4.1 ±0.2 }	19.5 ±2.7
33±1	4/4	4	{ 4.4 ±1.2 }	7.36	7.31	—	—
28±1	4/4	4	{ 2.12 ±0.92 }	7.32	7.44	—	—
25±1	17/17	5	{ 0.64 ±0.36 }	7.26	—	{ 4.7 ±0.4 }	9.3 ±2.1
25±1	4/4	4	{ 0.87 ±0.24 }	7.45	7.02	—	—

fibrillation following calcium chloride infusions. The remaining 35 per cent escape fibrillation and terminate in asystole (cardiac contracture). The amount of calcium chloride necessary to produce asystole in the latter is almost twice that required to cause fibrillation in the former. These results on normothermic dogs are in agreement with those previously reported for similar experiments by Hoff *et al.*²² In contrast to the normothermic dogs, calcium infusions produce ventricular fibrillation in *all* hypothermic dogs. And, furthermore, the amount of calcium required to produce fibrillation diminishes as hypothermia progresses, such that at 25° fibrillation may be caused by one-ninth the amount required at normal temperature. Terminal plasma calcium levels (heart samples) in the normothermic group are greater than the pre-infusion levels by a factor of 5, and in the hypothermic group by a factor of 2. These results indicate clearly that at 25° the dog's heart is several times more sensitive to exogenous calcium than at normal temperature, and from the available data the increased sensitivity is a progressive phenomenon.

Investigation of the pH factor on sensitivity to exogenous calcium in hypothermia reveals that if such a factor exists it is of a magnitude not revealed by the relatively crude technique employed. The artificially respired dogs with terminal pH levels in the normal range are no less sensitive than acidotic dogs. The mean pH of 7.26 represents a less extreme degree of acidosis in the hypothermic state than was encountered in earlier experiments where spontaneous respiration prevailed, but despite this the most acidotic dog (pH 7.12) required the largest infusion to produce fibrillation of any dog in the group. Thus maintenance of an approximately normal pH throughout cooling is without apparent effect on the sensitivity of hypothermic dogs to exogenous calcium (last group shown in table III). Similarly when calcium chloride is administered to normothermic dogs previously rendered acidotic by administration of CO₂ to the inspired air (mean pH < 6.9) it is found that such animals do not differ in sensitivity to calcium from the normothermic controls. It appears therefore that blood pH is not a factor determining the amount of calcium required to produce fibrillation in either normo- or hypothermic dogs.

The electrocardiographic changes following calcium infusions in both normo- and hypothermic dogs are similar to those described by Hoff *et al.*,²² and here shown in figures 5 and 6. It is of interest that one of the early changes in normal animals is the appearance of an upward deflection of the earliest portion of the S-T segment, similar to that described for some hypothermic dogs and resembling injury potentials. In hypothermic dogs possessing such an ECG the early S-T elevation is immediately augmented by calcium infusions. The significance of these observations remains obscure.

That the myocardial responses to exogenous calcium as just described are specific for calcium is substantiated in part, at least, by substitution of isotonic saline or isotonic potassium chloride. Volumes of the former in excess of any calcium chloride infusions employed produced no distinct ECG changes. Potassium chloride infusions in small series of normo- and hypothermic animals yielded different characteristic responses from those described for calcium. The average lethal dose was 2.7 mEq. of potassium per kg. for normothermic, and 1.8 mEq. per kg. for hypothermic dogs. The difference is not statistically significant for these small groups.

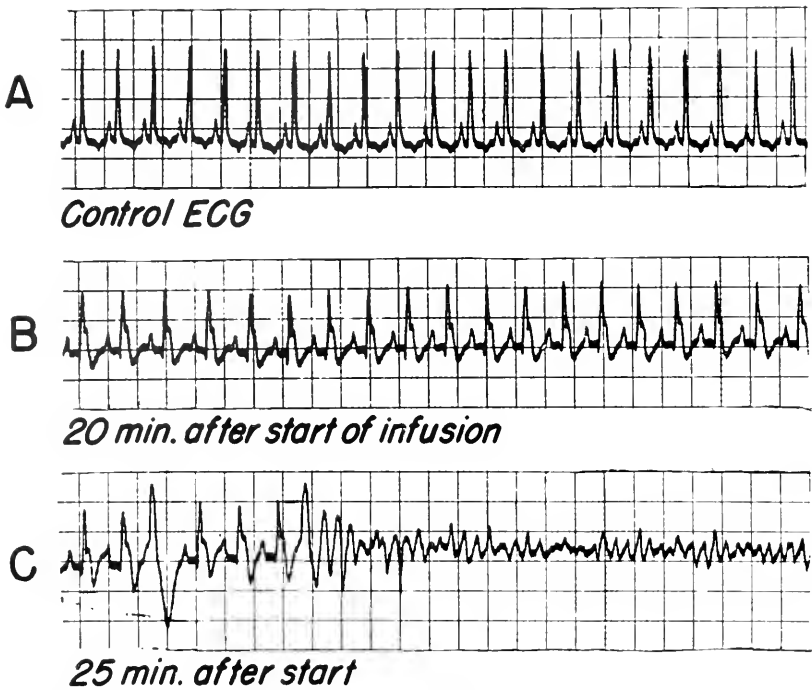


FIG. 5.—Progressive ECG changes in normothermic dogs during continuous infusion of isotonic calcium chloride.

The electrocardiographic changes are similar in the two groups, but distinct from those for calcium. There occurs a progressive decrease in heart rate followed by a widening of the QRS complex, inversion and increase of the T-wave, and shortening of the S-T interval to complete extinction. The preterminal ECG complex consists of a wide upward QRS passing into a similar wide deflection below the base line. The terminal event is diastolic asystole in both hypothermic and normothermic dogs. Thus hypothermia appears not to alter the sensitivity of the heart to exogenous potassium.

With respect to the ECG phenomena associated with calcium infusion, the so-called injury current differs only in magnitude from that which develops in acidotic hypothermic dogs. It is tempting to think that the two have a common origin, related to a disturbed calcium balance of the myocardium. That both are associated with augmented excitability appears established.

The question regarding the role of temperature in the establishment of lower ventricular thresholds, the deviation of the S-T segment, and the electrolyte balance of the myocardium is not fully answered. Certainly a temperature factor is not conspicuous in relation to these phenomena during progressive hypothermia, being overshadowed by the factor of pH. Without additional evidence one might conclude that temperature *per se* was without influence. The observations which suggest that temperature may not yet be ruled out are: (a) Reversibility of the conditions leading to fibrillation, and the S-T segment change, are not readily achieved by a simple

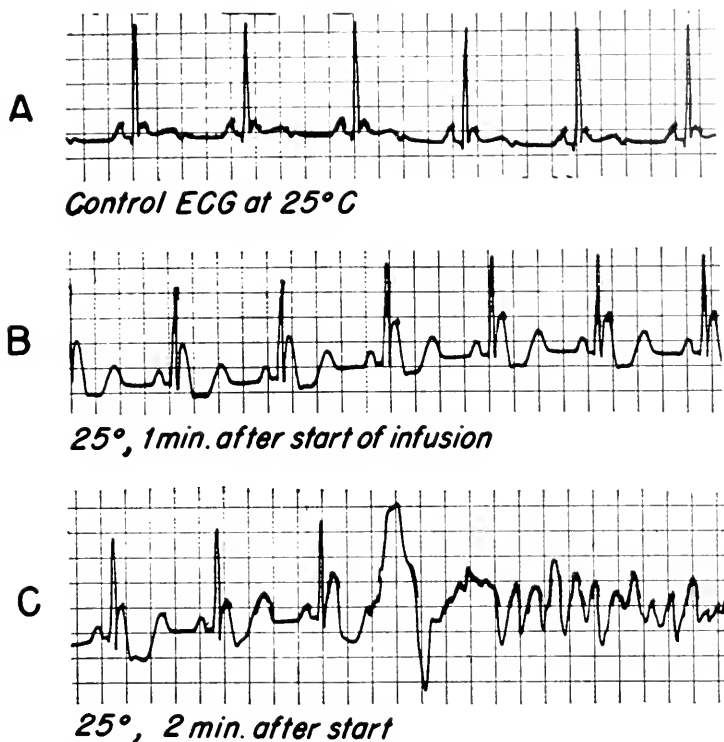


FIG. 6.—Progressive ECG changes in hypothermic dogs during continuous infusion of isotonic calcium chloride.

pH reversal at low temperature; but the latter, at any rate, appears so at normal temperature.¹⁷ (b) Reversal of the ECG to normal is readily accomplished by rewarming even before pH restoration is advanced (unpublished data). (c) Several investigators have commented upon the fact that spontaneous fibrillation rarely if ever occurs above the 25° to 26° temperature. First note was made in connection with "rewarming" death¹ which is also fibrillary, and which to date has not been seen above 25°. During cooling, fibrillation is anticipated in a proportion of dogs when the 25° to 26° level has been reached or passed.^{1, 9, 12} The development of an acidotic state, too, is gradual and progressive, and one may with equal logic propose that the critical pH for fibrillation is reached at 25° under the experimental conditions described. Despite this, our own impressions favor temperature, as the important factor. (d) The degree of acidosis required to produce diastolic threshold changes in normothermic dogs is greater than that in hypothermic (6.5–6.9 vs. 7.1–7.2).⁶ (e) The increased sensitivity to exogenous calcium is apparently a temperature effect.

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DISCUSSION

Dr. F. John Lewis: My comments concern Dr. Hegnauer's presentation, and are based on the work of my associate, Dr. Niazi.

I will describe our use of carbon dioxide with an open respiratory system to reduce the incidence of ventricular fibrillation. Superficially, the figures I am about to show you appear to contradict some of the work that Dr. Hegnauer presented. Perhaps the most important differences between Dr. Hegnauer's experiments and ours concern the type of respiration used. We did not depend upon spontaneous

respiration in our experiments, and in all of them we have had to struggle to avoid respiratory alkalosis rather than respiratory acidosis.

Dr. Niazi started his work in rats and showed that he could reduce their temperature to about 15° C. before cardiac standstill occurred. Rather than discard these rats after the heart had stopped he tried to revive them and found that he was almost uniformly successful in doing so. Because his experiments were so promising, we bought him a much more efficient respirator; and with this respirator, he lost all his rats. When he used 5 per cent carbon dioxide in oxygen rather than oxygen alone with the more efficient respirator, he was able to save most of them.

The change in pH that occurred in dogs respired with this respirator during cooling are shown in figures 1 and 2. With oxygen alone the pH rose to high levels, but it remained relatively constant at levels slightly below normal when 5 per cent carbon dioxide was used.

The efficacy of using carbon dioxide during cooling is shown in two ways. First, animals have been cooled to levels below 10° C. without ventricular fibrillation; and second, there appears to have been a reduced tendency to ventricular fibrillation when operating on the cold heart.

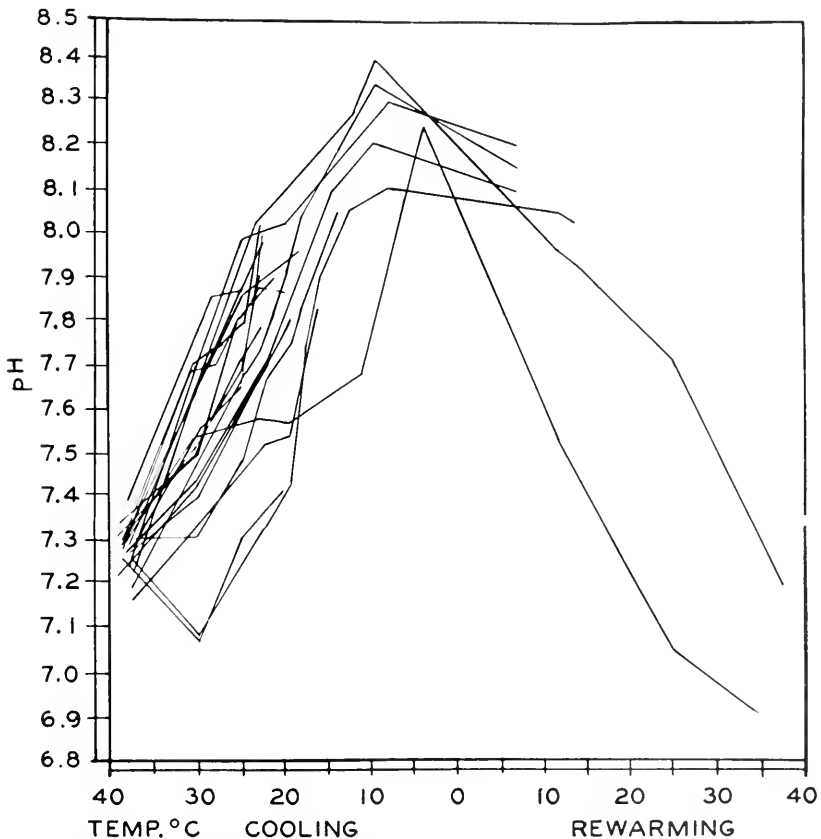


FIG. 1.—Changes in blood pH while cooling dogs which were breathing oxygen with an artificial respirator. (By permission of Surgery, Gynecology and Obstetrics.)

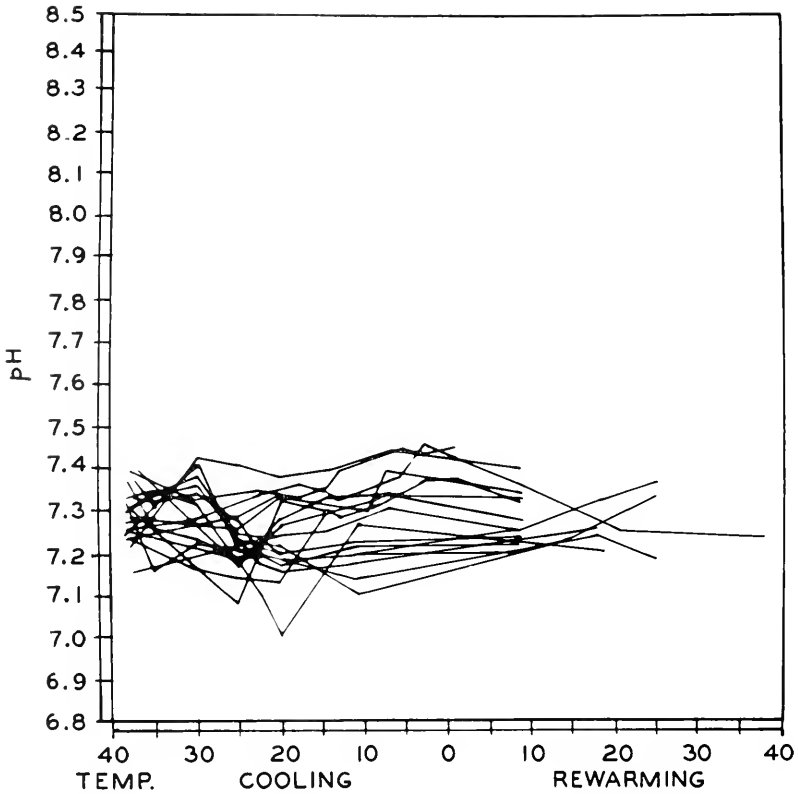


FIG. 2.—Blood pH in dogs breathing a mixture of 5 per cent carbon dioxide in oxygen. (By permission of Surgery, Gynecology and Obstetrics.)

Resuscitation of dogs from temperatures below 10°C . has been impossible on oxygen alone. With the high pH's that occur most of the animals fibrillated at low temperatures. Some of them had a continued heartbeat, especially the younger ones. Here age differences complicate matters, but in any case, none of them survived the complete experiment.

When 5 per cent carbon dioxide in oxygen was used with the same respiratory rate (about 13 respirations per minute), the pH was maintained at a level slightly below normal. All the young dogs could be cooled to below 10°C . with the heart continuing to beat and without fibrillation, though they could not be resuscitated. The adult animals, if given only 5 per cent carbon dioxide, still developed ventricular fibrillation, though at lower temperature levels. By giving them 10 per cent carbon dioxide, however, nine out of twelve were cooled to 10°C . or lower without fibrillation, though they still were not resuscitated. In contrast to this, with oxygen alone all adult animals fibrillated at temperature levels between 19° and 23°C ., as other investigators have shown.

When the pH was raised to a high level at 20°C . through adding oxygen as shown in figure 3, 45 per cent of 38 animals went into cardiac standstill. The rest went into ventricular fibrillation or their hearts continued to beat. Those which fibril-

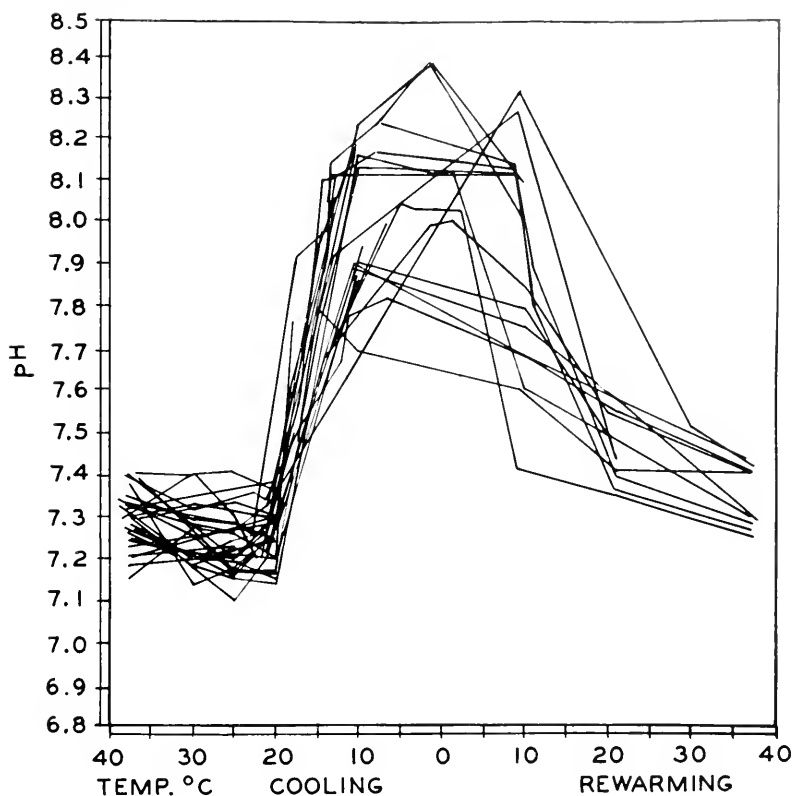


FIG. 3.—Blood pH in dogs, illustrating how the pH shifts to a high level when the respiratory mixture is changed from 5 per cent carbon dioxide in oxygen to oxygen alone at a body temperature of 20° C. (By permission of Surgery, Gynecology and Obstetrics.)

lated and those which had a continuing heartbeat were not resuscitated, but of those which went into standstill and were cooled to below 10° C., 76 per cent were resuscitated and lived indefinitely. At normal temperatures a similar shift in pH causes a much higher incidence of ventricular fibrillation. Not only are pH levels important but shifts in pH too, may be crucial. I think that the use of spontaneous respiration during cooling with change to artificial respiration at low temperatures just before doing a thoracotomy, a technic employed by Hegnauer, may produce a detrimental shift in pH.

These next experiments showed a reduced incidence of ventricular fibrillation during operations on the heart at 18° C. if 5 per cent carbon dioxide was employed. Right ventriculotomy was done on 9 consecutive dogs without fibrillation. In earlier experiments fibrillation had occurred even with the carbon dioxide; when the technique was mastered, however, this was avoided. Careful technique is a very important factor in reducing the incidence of ventricular fibrillation.

In humans the results have been similar; pH levels above 7.45 have been dangerous. To begin with we used an ordinary anesthesia machine and oxygen alone. Among the first 32 patients in which atrial septal defects were repaired under

direct vision, using hypothermia, 11 (33 per cent) had ventricular fibrillation. By using 5 per cent carbon dioxide in oxygen and an artificial respirator, we have had only one case of ventricular fibrillation in twelve operations. All except one of the fibrillators in which blood chemistries were measured had pH's above 7.45 during cooling.

Profound cooling has also been successfully carried out in monkeys. Using carbon dioxide, it has been possible to cool monkeys to temperatures below 10° C. without ventricular fibrillation and successfully resuscitate them. So far we have cooled five monkeys and lost only one. One of them had his cardiac action arrested for 56 minutes and was perfectly normal after he recovered.

Dr. B. F. Hoffman: Several other people who have studied hypothermia have noticed this same electrocardiographic change which Dr. Hegnauer mentioned. The electrocardiogram reveals an elevated junction, J, and ST primarily below the base line.* I am impressed by the potassium and calcium interrelationships which Dr. Hegnauer has mentioned, and I think that we could account for this type of ECG on the basis of the electrolyte abnormalities which he has found. The normal cardiac action potential reveals a prominent plateau prior to repolarization. In the ventricle the effect of increased extracellular calcium is to change the configuration of the action potential so that the plateau is abolished.

If we assume that some of the fibers in the ventricular myocardium are more strongly influenced by calcium than others and thus have some fibers repolarizing along the normal time course and others along a "high calcium" time course, and we take an electrocardiogram from that ventricle, we will get a tracing exactly like the ones which he has recorded. This is a known effect of high calcium on the ventricular muscle. I don't think we should imply, on the other hand, that this ECG configuration would result only from high extracellular calcium, because localized anoxia also abolishes the plateau from the ventricular action potential, and with anoxia of 15 or 20 minutes' duration one sees an action potential configuration similar to that produced by Ca excess. With some areas of the heart more anoxic than others, we would again have a situation obtaining in the cells which would result in an elevated J, a depressed ST segment and very deep T wave which tends to stay on one side of the base line.

I think that if this tracing recorded from dogs prior to fibrillation has any general significance, it is probably that some influence, either anoxia or change in calcium and potassium fluxes across the cell, has progressed further in some localized area than in other parts of the heart.

I might emphasize that whenever we do not have a uniform condition obtaining in the ventricle, the heart is much more susceptible to fibrillation than when all of the cells follow a similar time course of depolarization and repolarization.

Dr. Gollan: We have heard from different investigators that a hypothermic heart whose coronary circulation is perfused with oxygenated blood, does not lose potassium, continues to beat at lower temperatures and is protected against ventricular fibrillation. These phenomena, especially the remarkable tolerance to myocardial ischemia, can be observed in the dog whose venous blood is passed through a small oxygenator and returned into the left subclavian artery.

* Dr. Hoffman illustrated this and the following ECG configurations at the blackboard.

If the main trunk of the left coronary artery is occluded for 2 minutes all normothermic dogs develop ventricular fibrillation (fig. 1). Since the function of the heart and lungs is taken over by the instrument, the rhythm of the heart is of little concern. Ventricular fibrillation has lost its nightmarish aspect and is just a cosmetic defect of the electrocardiogram! After 2 hours, ventricular fibrillation can be stopped by electrical shock and the pump-oxygenator can be turned off.

In hypothermic dogs with by-pass of heart and lungs the period of complete coronary occlusion can be progressively prolonged (fig. 2). At 23° C. the empty, beating heart can be deprived of coronary flow for 25 minutes without ventricular fibrillation. Longer periods of coronary occlusion at lower temperatures were not tested in this series of experiments. After occlusion of the aorta the heart rate decreases in a linear fashion with time (fig. 3) and the electrocardiogram shows signs of severe ischemic injury (fig. 4). This electrical injury is of a functional nature only, because after release of the aortic occlusion and after perfusion with oxygenated blood, normal electrocardiogram and heart function can be established. The degree and dynamics of myocardial ischemia and the rapidity of its recovery can be demonstrated by measuring myocardial oxygen tension polarographically with a platinum electrode (fig. 5).

It should be pointed out that the changes recorded are not absolute ones because no corrections are made for temperature and pH effects. In this experiment clamping of the ascending aorta was followed by a gradual decrease in myocardial oxygen tension until about half of the available oxygen was used up after 6 minutes. After release of the aorta it took the heart muscle about one minute only to return to its previous baseline of oxygen tension. The slowing of the heart rate and the decrease of amplitude of excursions during the ischemic period can also be

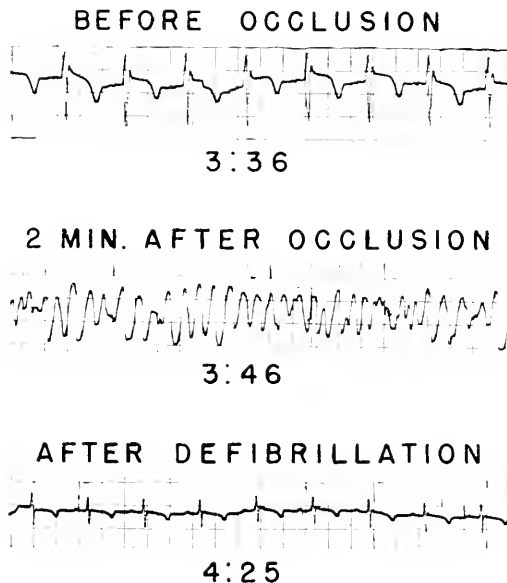


FIG. 1.—Temporary occlusion of main trunk of left coronary artery during partial by-pass of heart and lungs (36° C.).

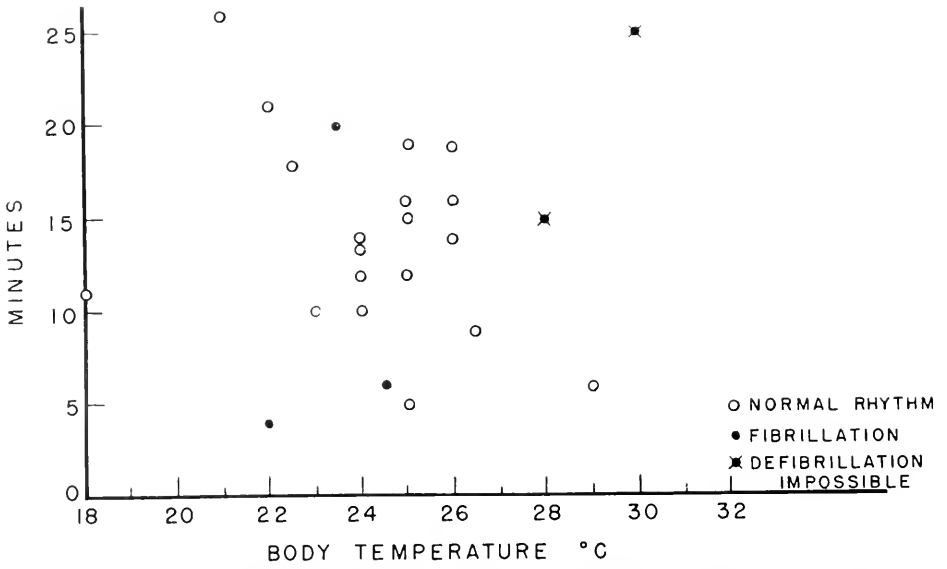


FIG. 2.—Duration of aortic and pulmonary occlusion in hypothermic dogs during by-pass of heart and lungs.

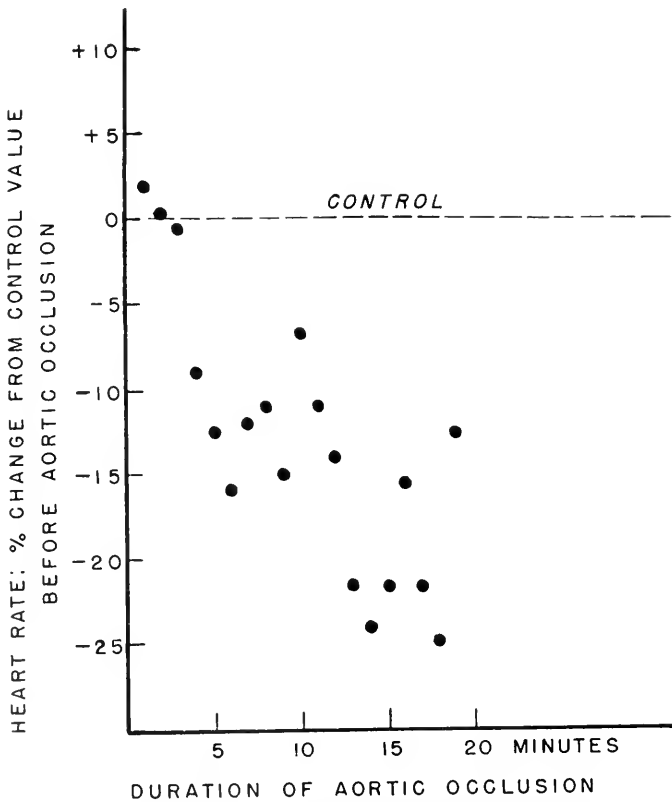


FIG. 3.—Mean per cent change in heart rate of hypothermic dogs during by-pass of heart and lungs, aorta and pulmonary artery occluded.

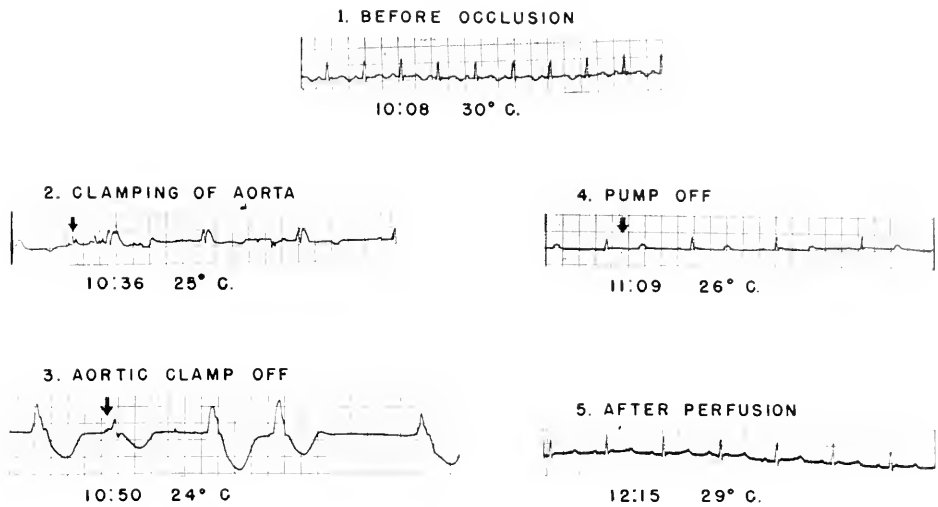


FIG. 4.—Prolonged clamping of aorta and pulmonary artery in hypothermic dogs by blood cooling.

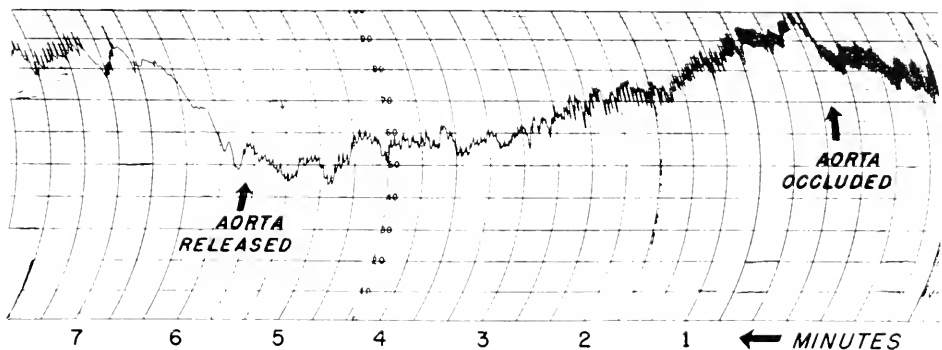


FIG. 5.—Myocardial oxygen tension after coronary occlusion (26°C , extracorporeal oxygenation).

recorded. On release of the aorta the heart goes into arrest which is probably due to release of potassium into the coronary vascular bed and after a minute of perfusion with oxygenated blood the heart starts to beat again. Such periods of complete coronary occlusion can be repeated at will and in one experiment the myocardium was subjected to three consecutive occlusions of 20 minutes each at 24°C . After each occlusion the oxygen tension rose in 1 to 2 minutes to its original level and the heart could tolerate again another prolonged period of ischemia. If the aorta was occluded before the oxygen tension had returned to normal, ventricular fibrillation developed which did not respond to electric shock.

The clinical applications of this remarkable ischemic tolerance of hearts perfused with oxygenated cooled blood in the surgical repair of mitral and aortic disease is evident.

Dr. A. Riberi (with H. B. Shumacker): In 1954 a brief note appeared in the foreign letters section of the J.A.M.A. stating that Lian and his associates found

that blocking the sino-auricular node permitted the performance of right ventricu-
lotomy without ventricular fibrillation in dogs under drug-induced "artificial hiber-
nation."¹ Knowing of this work prior to its publication, a group of us at Indiana
University began some studies in December 1953 which have proved informative.
All of these experiments were performed upon dogs anesthetized with intraven-
ously administered thiopental sodium, intubated, and hyperventilated with oxygen
by a mechanical insufflation apparatus operating from 35 to 45 times per minute.
All were cooled by immersion in a bath of water and cracked ice. At the completion
of the operative procedure they were rewarmed to a temperature of approximately
36 degrees in a bath of warm water.

We found that injection of procaine into the area of the superior vena caval-
atrial junction (fig. 1) produced slowing of the heart, some drop in blood pressure,
and alterations of the electrocardiographic P wave, suggesting sino-auricular node
blockade.² In roughly 70 per cent of the animals the P wave disappeared (fig. 2)
and in the remainder it showed reduction in voltage or inversion. In a series of 10
animals the average reduction in pulse rate was from 67 to 46, the blood pressure
from 146/110 to 125/78, and the duration of the effect as measured by P wave
changes a little less than 20 minutes (table I). In 10 normothermic dogs the aver-
age reduction in pulse rate was from 143 to 91, the blood pressure from 174/122 to
165/98, and the duration of effect as measured by P wave alterations was approxi-
mately 10 minutes. We gained the impression that the cardiac rhythm was more
stable and the heart less irritable on external manipulation after such injection. In
the hypothermic animals the heart also appeared pinker.

The apparent decrease in myocardial irritability proved to be correct when sub-
jected to the test of other manipulative procedures. In moderately hypothermic ani-

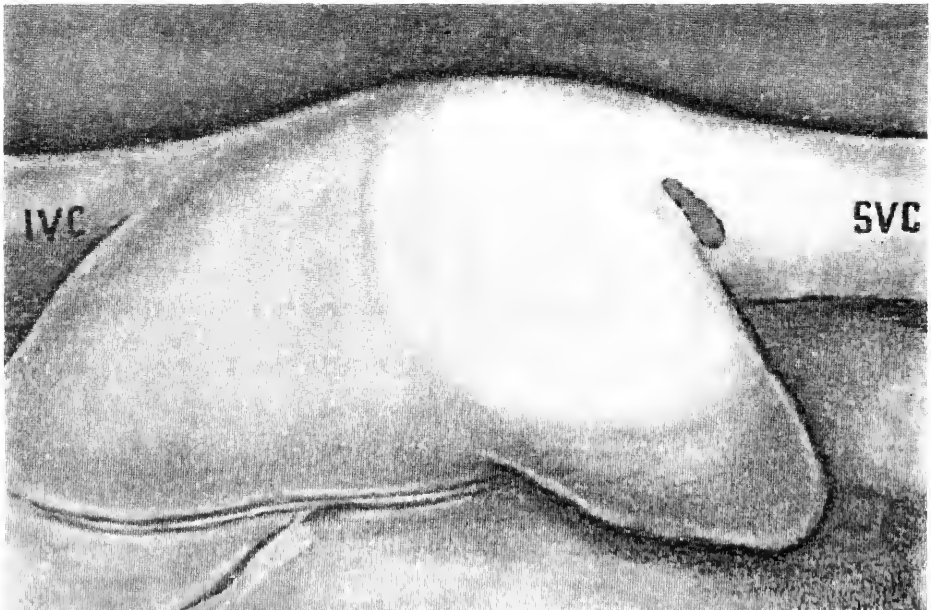


FIG. 1.—Drawing showing area of procaine infiltration. From Riberi, *et al.* (Surgery, Nov., 1955.)

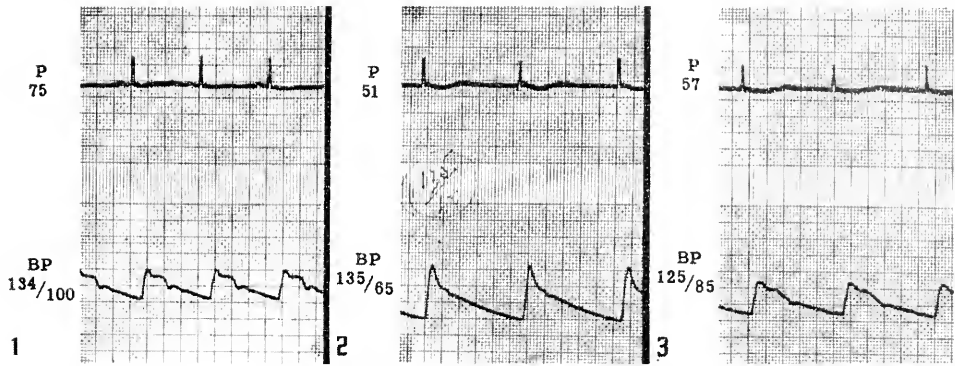


FIG. 2.—Effect of procaine injection of superior vena caval-atrial junction upon electrocardiogram (lead 2), pulse and blood pressure in hypothermic dog, temperature 27 degrees. From Riberi, *et al.* (*Surgery*, Nov., 1955.)

mals³ the stimulus of very rough external manipulation or vigorous massage of the ventricular septum by a finger passed into the right ventricle through the right atrium and tricuspid valve resulted in ventricular fibrillation in all control animals and in none protected by procaine injection (table II). When animals were stimulated by right ventriculotomy, or by ventriculotomy and placement of sutures in the ventricular septum, similar results were obtained. Ventricular fibrillation occurred in all control animals and in none of those injected with procaine. The series of animals was not enlarged because the results were so conclusive and because Radigan, Lombardo, and Morrow, who repeated our experiments, obtained the same results.⁴ When the experiment was carried out upon dogs cooled to a lower temperature, only 56 per cent of the control animals developed fibrillation.⁵ Those with procaine blockade showed a remarkable degree of protection. Fibrillation was prevented in 94 per cent.

We have also investigated the influence of the extrinsic nerve supply to the heart on the incidence of ventricular fibrillation⁵ (table III). In moderately cooled dogs subjected to a 10-minute period of venous inflow occlusion, right ventriculotomy and placement of sutures in the ventricular septum, upper dorsal and stellate ganglionectomy protected all animals from ventricular fibrillation. The use of in-

TABLE I

AVERAGE PULSE, BLOOD PRESSURE AND ELECTROCARDIOGRAPHIC CHANGES IN DOGS WITH PROCAINE BLOCK OF ATRIAL-SUPERIOR VENA CAVAL AREA

(Adapted from *Surgery*, Nov., 1955.)

No. of dogs	Pulse rate		Blood pressure		Alteration in P wave	Duration of P wave changes
	Before	After	Before	After		
10.....	143	91	174/122	165/98	Disappeared, 70% Altered, 30%	9.7
Normothermic animals						
10.....	67	46	146/110	125/78	Disappeared, 70% Altered, 30%	18.2
Hypothermic animals						

TABLE II

EFFECT OF PROCAINE BLOCK OF ATRIAL-SUPERIOR VENA CAVAL AREA UPON INCIDENCE OF VENTRICULAR FIBRILLATION IN HYPOTHERMIC DOGS

(Adapted from Surgery, Nov., 1955.)

No. of dogs	Stimulation: Rough external manipulation or finger massage of ventricular septum						
	Untreated controls			Treated (procaine block)			
	Temp. range	Inflow occlusion: time, min.	Per cent fibrillation	No. of dogs	Temp. range	Inflow occlusion: time, min.	Per cent fibrillation
6	24.5-27	4.8	100	8	23.5-27.5	10.4	0
	Stimulation: Right ventriculotomy or right ventriculotomy and placement of sutures in ventricular septum						
6	27-28	4.2	100	11	24.6-28.3	10.4	0
16	19.5-22.5	8.2	56	16	19-22.5	10.3	6

travenous or intra-atrial Arfonad, in sufficient amount to produce an average blood pressure decrease from 153/11 to 60/26, was similarly effective, the incidence of fibrillation being only 13 per cent.

Bilateral section of the cervical vagosympathetic trunks, which interrupts vagal but not sympathetic fibers to the heart, resulted in almost as high a percentage of fibrillation as occurred in untreated control animals. On the other hand, as Montgomery and his associates reported,⁶ stimulation of the right vagus lowered the incidence of fibrillation. If we exclude three animals in which vagal escape occurred, fibrillation was noted in only 17 per cent. When bilateral upper dorsal sympathectomy and stellate ganglionectomy were combined with division of the cervical vagosympathetic trunks, 40 per cent of the animals developed fibrillation. The observation made in animals in which the vagal nerves were divided distal to the caudate ganglion, and distal to the origin of the depressor nerves is difficult to understand. Only 20 per cent of these animals had fibrillation.

TABLE III

EFFECT OF EXTRINSIC CARDIAC INNERVATION UPON INCIDENCE OF VENTRICULAR FIBRILLATION IN DOGS COOLED TO APPROXIMATELY 26° TO 27° C. AND SUBJECTED TO VENOUS INFLOW OCCLUSION FOR 10 MINUTES, RIGHT VENTRICULOTOMY, AND PLACEMENT OF SUTURE IN VENTRICULAR SEPTUM

(Surgery, Nov., 1955)

Type of alteration of nerve supply	No. of dogs	Per cent developing fibrillation
Bilateral upper dorsal sympathetic and stellate ganglionectomy...	15	0
Injection of intra-atrial or intravenous Arfonad.....	15	13
Bilateral section of cervical vago-sympathetic trunks.....	15	80
Stimulation of the right vagus.....	15	33 (entire group) 17 (excluding 3 in which vagal escape occurred)
Bilateral division of vagi distal to caudate ganglia.....	15	20
Bilateral upper dorsal sympathetic and stellate ganglionectomy and bilateral division of cervical vago-sympathetic trunks.....	15	40

Thus far it seemed clear that certain measures almost completely prevented ventricular fibrillation in moderately hypothermic dogs stimulated by maneuvers which in control subjects invariably were associated with fibrillation. Such measures include procaine blockade of the superior vena caval-atrial junction, sympathetic interruption or blockade, and vagal stimulation.

These experiences proved of some value in the treatment of ventricular fibrillation induced by coronary air embolism in moderately hypothermic dogs.⁷ It was our experience that conversion could be successfully accomplished in all animals by making the fibrillation strong with the intracardiac injection of a weak solution of epinephrine, clamping the ascending aorta, eliminating the air from the coronaries by massage, and, if necessary, by incising terminal branches of arteries with entrapped air, and by the application of three successive electric shocks using 170 volts and a duration of one-tenth of a second. In 10 control animals fibrillation occurred in all (table IV). In one, fibrillation recurred once and in two others, three times. In one of the latter, procaine blockade preceded the final successful conversion. In six animals, injection was carried out after the onset of fibrillation and in none did fibrillation recur. In four animals, procaine blockade was accomplished before the expected onset of fibrillation. Fibrillation did not occur in one and it did not return after conversion in the other three. When the same experiments were repeated with the added procedure of right ventriculotomy and placement of sutures in the septum, fibrillation developed in all (table V). The fibrillation did not recur, however, in any animal treated by procaine injection before the expected

TABLE IV

EXPERIENCES WITH TREATMENT OF CORONARY AIR EMBOLISM AND VENTRICULAR FIBRILLATION IN THE HYPOTHERMIC DOG BY CARDIAC MASSAGE, INTRACARDIAC EPINEPHRINE, AND ELECTRIC SHOCK
(Surgery, Nov., 1955)

Sinoauricular node injection prior to expected onset of fibrillation							Remarks
No. of dogs	Temp. of dogs in °C.	Average volume air injected (in cc.)	Average interval between injection of air and onset of fibrillation in minutes	Average duration of fibrillation in minutes before institution of massage	Average period of massage in minutes necessary for clearing coronaries of air	Average duration of fibrillation in minutes	
4.....	26-26.5	4.9	4.8	2.5	2.1	9	One did not fibrillate; others resuscitated.
Sinoauricular node injection after onset of fibrillation							Remarks
6.....	26-26.5	3.9	2	3.3	5.3	9.8	
No sinoauricular node injection ^a							Remarks
10.....	25-26.5	4.2	0.8	4.75	2.3	13	

^a One had injection of the sinoauricular node after fibrillation recurred 4 times.

TABLE V

EXPERIENCE WITH TREATMENT OF CORONARY AIR EMBOLISM AND VENTRICULAR FIBRILLATION IN HYPOTHERMIC DOGS SUBJECTED TO RIGHT VENTRICULOTOMY AND PLACEMENT OF SUTURES IN VENTRICULAR SEPTUM BY CARDIAC MASSAGE, INTRACARDIAC EPINEPHRINE, AND ELECTRIC SHOCK

(Surgery, Nov., 1955)

No. of dogs	Temp. of dogs in ° C.	Sinoauricular node injection after onset of fibrillation					Remarks
		Average volume air injected (in cc.)	Average interval between injection of air and onset of fibrillation in minutes	Average duration of fibrillation in minutes before institution of massage	Average period of massage in minutes necessary for clearing coronaries of air	Average duration of fibrillation in minutes	
4.....	26-27	4.1	2.2	5.6	2.2	7.5	Fibrillation in all; no recurrence of fibrillation; all resuscitated.
4.....	25.5-27	4.4	1.1	5.2	2	27	Fibrillation in all; fibrillation recurred 3 times in one, 10 times in another; ^a all resuscitated.

^a Before the last successful defibrillation sinoauricular node was injected.

onset of fibrillation, while it occurred repeatedly in half the others. In one, fibrillation developed 10 times and lasting conversion followed procaine injection.

Using the information derived from these experiments, we were able to produce and repair ventricular septal defects in a series of dogs without mortality.⁸

Recent experiments have demonstrated that the period of safe venous inflow occlusion in moderately hypothermic dogs can be markedly prolonged by perfusion of the coronary arteries, or the coronary and carotid arteries, with oxygenated blood.⁹ These animals were cooled to a body temperature of from 24° to 28.5° C. Of 12 dogs with coronary perfusion subjected to venous inflow occlusion of from 25 to 29.5 minutes, an ultimate normal recovery was made by 11. The exceptional animal died of hemorrhage. One of the animals exhibited hind leg weakness for two days, five were stuporous the day following surgery, and one for three days. The behavior and function of all, however, appeared normal thereafter. Fifteen animals with coronary and carotid artery perfusion were subjected to right ventriculotomy and venous inflow occlusion of from 26 to 35 minutes. All recovered without any evidence of cord or brain damage. Three, however, died later of hemorrhage. One with an occlusion period of 38 minutes died after five days of stupor. In some animals in both groups ventricular fibrillation occurred after release of caval occlusion but in all conversion was easily accomplished. All had been protected by procaine blockade of the superior vena cava-atrial junction. Transient neurologic sequelae were noted in some of the animals with coronary perfusion alone and in none with coronary and carotid perfusion subjected to comparable periods of venous inflow occlusion. The fact that eventual recovery took place in

both groups, however, would indicate that prompt restitution of cardiac function after prolonged venous inflow occlusion is more important in ultimate recovery than is brain perfusion. Procaine blockade and coronary artery perfusion with oxygenated blood clearly permits a greatly increased period of safe caval occlusion. Additional safety is derived from simultaneous carotid artery perfusion.

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Dr. Jean Cahn: In 1953 two brief papers on the prevention of ventricular fibrillation by sino-atrial blockade appeared from our laboratory.^{1, 2}

What we call sino-atrial blockade is the injection into the sinus node of a 1 per cent solution of lignocaine; it is very important not to replace lignocaine by procaine, because the duration of anesthesia produced on the sinus node is longer with lignocaine than with procaine. We have done experimental work on dogs and monkeys with an arrest of the blood circulation longer than 25 to 30 minutes. In the data reported by Dr. Shumacker the arrest of the blood circulation is not longer than 15 minutes. We have proof that procaine cannot act longer than 12 to 15 minutes; it is destroyed rapidly by procaine esterase. So, even though procaine gives protection during the first part of the operation, ventricular fibrillation may occur during the cardiac massage if the duration of the arrest of the circulation is longer than 17 minutes.

We must inject lignocaine not only in the junction area between the superior vena cava and the auricle; that is not sufficient. I could show you that it is possible to stop the heart completely with an injection of lignocaine 1 per cent at the junction between the superior vena cava and the right auricle, but this does not avoid ventricular fibrillation if the duration of the arrest is longer than 20 minutes. It is

necessary to inject lignocaine in the sinus node from the superior vena cava to the inferior vena cava in three or four points. With this method we obtain, first, a very marked bradycardia; second, sometimes brief cardiac arrest (30 seconds); third, a nodal rhythm. It also seems that the contraction of the ventricle begins only after the acute dilation of the auricle. There is a mechanical contraction which is the result of the autonomic rhythm imposed to the heart after the sino-atrial blockade.

The benefit, I think, is not only in the diminution of the cardiac excitability, but in the slow heart rate. When the heart is clamped, the metabolism of the heart is very different in spite of hypothermia: the metabolism of the heart is an anoxic metabolism; and the immediate reaction of the heart in those anoxic conditions is first, the outpouring of potassium, second, the use of glycogen as the only emergency fuel. It seems that the heart cannot use more than a certain quantity of its own glycogen; the slower the heart rate the more economical is the use of glycogen and the longer can be the duration of the arrest of the circulation.

On the other hand, in the same period of arrest of the blood circulation we still have coronary flow. We were surprised to see that during 12 to 14 minutes we still had coronary flow in spite of the occlusion of both vena cava and azygos. Certainly the coronary flow is diminishing gradually during those 12 to 14 minutes, and after 15 minutes of clamping the coronary flow is about 1 per cent of its value at the beginning of the clamping.

At the same time there is a reduction in the oxygen supply, and there is also a reduction in all the metabolite supply to the heart.

What are our results with this method in surface cooling and in artificial hibernation? In surface cooling we could arrest the blood circulation in dogs cooled between 23 and 24° C. during 22 and 30 minutes without any cases of ventricular fibrillation in 22 dogs. In artificial hibernation we did the same work, and the arrest of the blood circulation was also from 17 to 28 or 30 minutes. We have had about 2 per cent of ventricular fibrillation.

In 16 monkeys we did the same experiment in surface cooling and in artificial hibernation with exactly the same results: No cases of ventricular fibrillation in spite of 16 to 25 minutes of arrest of the blood circulation.

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REVIEW AND APPRAISAL OF PART III

CHANDLER McC. BROOKS AND BRIAN F. HOFFMAN

Throughout the Symposium, statements have been made which relate to the effect of cooling on the behavior of the heart and its responsiveness to stimuli. The major contributions to this subject, however, are to be found in the papers dealing with the action of hypothermia on the myocardium.

This brief summary has been written to call attention to and correlate statements made throughout the Symposium which are pertinent to the study of *Cardiac Irritability*, to consider and if possible resolve contradictions and to indicate various problems needing additional study. These three considerations will not be handled independently but will be treated under specific headings chosen for discussion from among the many presented. It is the opinion of the reviewers that these are the subjects most extensively dealt with in considerations of the action of hypothermia on heart function.

SPECIFIC THEMES AND CONCEPTS DISCUSSED

The oxygen supply and cardiac metabolism. One conclusion was definite and that is that in hypothermia the blood carries sufficient oxygen to the heart to meet its calculated requirement (Adolph, Friedman, D'Amato, Kao, Horvath and Spurr) as long as that organ continues to beat in a coordinated fashion and the oxygen can be released to the tissues (Fuhrman). Although there is an increase in anatomic dead space there is no evidence of difficulty in elimination of CO_2 due to a failure of gas transport (Severinghaus). Gross coronary flow is adequate (Berne, D'Amato) until fibrillation or asystole occur.

A second conclusion can also be accepted. Despite the adequacy of the oxygen supply metabolism cannot be said to proceed normally. It is of course reduced but more significant is the fact that imbalances develop. Whether or not these abnormalities are due to perfusion difficulties and transport between capillaries and cells cannot be decided on the basis of evidence presented but the reported changes in H ion, potassium, sodium and calcium levels, as well as in the efficiency of contractile processes, indicate that hypothermia has had a selective action. Normal balances and exchanges are not maintained.

Analysis of functional reactions. Subdivision of the reactions of the heart into individual, but interrelated processes, was stressed in several papers presented. The differential effect of temperature change has been employed in this type of analysis. Brown pointed out a number of examples of information thus obtained. Low temperatures slow the rates of all processes and modify the action of metabolites but this is not necessarily harmful until anoxia and chemical imbalances begin to develop. Circulatory failure is the limiting condition in hypothermia which initiates such anoxia and imbalance. The development of imbalances depends upon the temperature coefficients of the several processes involved in cellular function. Metabolic and rhythmical processes exhibit a Q_{10} of 3, contraction has a Q_{10} of 2, and physical processes such as diffusion of metabolites a Q_{10} of 1; thus, metabolic and rhythmical

activity decrease in hypothermia three times as fast as diffusion and twice as fast as contractility.

The selectivity of actions of B strophanthine and pressure on certain periods of the reaction cycle of the heart cells suggests that they amplify the alpha process of the contractile reaction and thus affect tensions developed. Temperature change can contribute to a much needed study of the specific physicochemical changes going on in the membrane which influence its polarization, repolarization and the associated reactions of the contractile process.

Similarly, hypothermia enhances the action of certain drugs and hyperthermia enhances that of others. This permits a degree of analysis of the specific action of these substances on cardiac tissue components and reaction processes (see Brown, Horvath, and Spurr).

The effects of hypothermia on the depolarization, repolarization and recovery of excitability described by Brooks for the whole heart and by Hoffman for cardiac cells provide other examples of subdivision of functional processes and the selective action of cooling. The analysis of the hypothermia-produced changes in the various phases of the cardiac cells' resting and action potential presented by Hoffman explains the modification of many of the grosser cardiac reactions. It should be pointed out that the temporary augmentation of the action potential at certain phases of cooling in both heart and nerve (Hoffman; Brooks and Koizumi) indicates that description of changes produced by hypothermia in simple Q_{10} and unidirectional relationships cannot be correct for all ranges of cooling (see Fuhrman).

A great deal of attention was paid to ion flux and partition. The ultimate balances maintaining at various temperatures were measured rather than the cyclical fluxes associated with the rising and falling phases of the action potential. Conclusions were somewhat contradictory but the majority of participants (e.g. Horvath, Spurr) claimed a slight loss of K from the cell in hypothermia. Shifts in Ca (an increased intracellular level) appeared to be more significant to the development of fibrillation (Hegnauer and Covino; McMillan, etc.). Such studies should be continued because our knowledge is inadequate at present.

Species and tissue peculiarities. In considering hypothermia and cardiac effects it is stated that in some species, hibernating animals for example, "the reaction rates of the various cellular processes have a better relative setting" although the temperature coefficients are the same. This hypothesis was employed by Brown to explain the cessation of cardiac rhythm at 13° C. in some species but not in hibernators. Such species differences may explain in part the discrepancies in results of tests of tolerance of the heart to cooling.

There are optimum temperatures for the development of tension by the cardiac tissues of different species (Brown, Lyman and Chatfield). Critical temperatures for conduction in nerves of different species differ (McQueen). Effect of temperature on various diametered nerve fibers differs.

Since it has been shown that cardiac cells differ (Hoffman, Brooks) specific studies of cellular susceptibilities to cold would add to the understanding of changes in behavior of the heart in hypothermia.

It is possible that protection of the ventricle from fibrillation in hypothermia by

blocking the sino-auricular node (Cahn, Riberi) might be due to a lesser depression of that pacemaker tissue. Continuation of a tendency to drive the heart at a rate faster than it could follow might explain the result. At least the observation provides an example of imbalance in specific tissue effects.

The work of Andjus, Lovelock & Smith, and that of Lewis in which cooling to extremely low levels and then rewarming was accomplished shows that fibrillation is not inevitable. Whether this is possible merely because of the small size of animals and hearts remains to be seen.

Diastolic threshold changes in hypothermia. In the experiments reported by Hegnauer, Gollan and others, the threshold changes to stimulation go either way, up or down. There are several possible ways in which this can be explained.

(a) *High thresholds.* (1) In experiments in which the heart is driven at a rate faster than the intrinsic frequency it is likely that the increase in threshold occurs at some low temperature where the Q-T interval tends to equal the cycle length (D'Amato) and thus diastolic levels of excitability are not attained.

(2) In Hegnauer's work (at least the earlier experiments reported in the literature) the measurements of "diastolic" excitability were made at a fixed point on the T wave. If it is true, as some work suggests (see Brooks), that at low temperature recovery of excitability lags behind repolarization in time, then a progressive increase in thresholds will be recorded with progressive cooling.

(b) *Low thresholds.* (1) It is interesting that in Hegnauer's work, in the animals which showed a decrease in thresholds and also a terminal fibrillation, the decrease in threshold began to appear at around 26° C. This is also the temperature at which a fairly rapid loss of resting potential and magnitude of action potential appears in studies of single fibers (Weidmann, Hoffman). A good case can be constructed for both a lowering of threshold, due to loss of resting potential, and failure of uniform conduction, due to loss of action potential amplitude. These factors could easily lead to fibrillation.

(2) The difference in electrodes and electrode placement may explain the difference in threshold of the ventricle to stimulation in the papers by Brooks and by Hegnauer and Covino. This same difference in technique may also explain the greater constancy obtained in experiments reported by Brooks and his associates. The observation by Hegnauer that animals in which cardiac threshold drops markedly on cooling are prone to fibrillate causes one to wonder if in these cases injury currents or currents originating from asymmetrical cooling (Berne: cooled coronary blood and fibrillation) might not have occurred and rendered the hearts vulnerable to fibrillation by the mechanism discussed by Brooks. Animals with more constant thresholds survived as did the animals of Gollan. A similar concept is in fact expressed by Hegnauer but the ECG asymmetry reported, though not thought to be due to injury, is not explained.

The fact that the cathode and anode can both stimulate (Durrer), and that at certain parts of the cycle the cathode is more effective while at other times the anodal stimulation predominates (Brooks, Durrer), should be taken into account. When the two electrodes are on different chambers of the heart (Hegnauer), multiple recordings are required to determine just what is happening.

Ion shifts in hypothermia. Gollan found decreased K/Na ratio in hearts of well-oxygenated hypothermic animals and in anoxic normothermic animals.

Szean found *positive* myocardial K balances during cooling.

Renkin perfused skeletal muscle and did not lose K.

Taylor perfused hypothermic heart and did not lose K.

Hegnauer had varying results, notably, an uptake of Ca by cold hearts and loss of K and H in fibrillators, and different electrolyte balances in non-fibrillators.

Other workers presented similarly contradictory results.

All of the studies reported are open to many criticisms. In the first place, it is not possible to accomplish any sort of electrolyte balance studies on tissues unless careful studies of *water shifts* are also made (see Horvath and Spurr). This applies to both intracellular and extracellular water.

The bulk of available evidence shows that cooling of any tissue is associated with a loss of intracellular K and uptake of Na. This change will be more marked in *active* than in quiescent tissue. On the other hand, if perfusion of the immediate extracellular space is poor, there will be two effects: 1) the local ion concentration will change in such a direction that additional shifts of electrolyte across the membrane will be minimized, and 2) the magnitude of transmembrane electrolyte shifts will not be accurately mirrored by the concentration in the plasma or perfusion medium. In addition, if there are significant water shifts, studies of perfusion fluid concentration will be of still less meaning.

These results are further complicated by two additional factors: (1) anoxia, and (2) membrane potential. It is reasonably certain, in both myocardium and other excitable tissues, that both *anoxia* and a *decrease* in resting potential alter the permeability of the membrane to both Na and K, so that Na is gained by the fiber and K is lost. Furthermore, anoxia can result in a change in membrane potential.

The results of Ca studies (Hegnauer, Lewis) seem reasonable. *If* the myocardial fibers lose K, they will certainly become more sensitive to Ca, as has been shown by many studies.

The significance of pH and CO₂ changes in hypothermia. The beneficial effects of alkalosis due to hyperventilation (Hegnauer) are open to considerable question (Lewis). Hegnauer states that too *much* alkalosis made fibrillation more likely, and that institution of artificial ventilation after development of acidosis did not prevent fibrillation. Lewis got his best results by preventing alkalosis with 10% CO₂, and others report the same findings. Hegnauer found that the blood pH had no effect on the sensitivity of hypothermic hearts to calcium infusion, and that coronary A-V potassium differences were not influenced by pH. Gollan states that at 23–24° C. the heart can be completely deprived of circulation for periods up to 20–25 minutes without fibrillation, and under these conditions the tissue pCO₂ must be very high and pH low.

All this makes one wonder if the various investigators have conducted studies of sufficient breadth. Perhaps the necessary approach, at both 38° C and lower temperatures, is to vary independently H ion concentration, pCO₂, O₂, HCO₃, etc. This has been done at normal temperature for certain hearts (Vaughn, Williams) and results show that [H⁺] and [CO₂] have some quite different effects.

Another line of work possibly indicated is to maintain some record of the CO₂

balance of the animal during the entire experiment. It is quite possible that, due to poor perfusion, the tissue levels of CO_2 are not accurately reflected by blood pH. The studies of Gollan, in which the circulation is maintained by a pump-oxygenator during cooling, suggest that perfusion may indeed be inadequate. Thus, he was able to cut off all coronary flow to cooled hearts (23 °) for 20 minutes without fibrillation, and when perfusion was resumed cardiac pO_2 returned to normal. This period of anoxia can be repeated. On the other hand, if the coronaries were reoccluded too soon after the first period of anoxia, irreversible fibrillation supervened.

In this respect, the use of a pump-oxygenator, and independent control of H and CO_2 seems indicated in intact animals. In addition, further study of isolated cardiac tissues is certainly suggested. It is perhaps significant that in isolated preparations (where perfusion is presumably adequate) arrhythmias are *not* seen during slow cooling.

Cardiac efficiency in hypothermia and rewarming. Although it is generally agreed (D'Amato, Berne, Adolph, Kao, etc.) that the heart's need for oxygen is satisfied during hypothermia some inefficiency is reported (McMillan, Swan). The reduction in cardiac and body metabolism permits the heart to supply itself and other tissues with needed oxygen (Horvath and Spurr; Rosomoff). No oxygen debt is incurred during hypothermia. The increase or decrease of cardiac efficiency appears to be of little significance in hypothermia but on rewarming (Swan) an efficiency of cardiac action capable of meeting the increased body needs is not immediately developed and rewarming may entail its own specific hazards.

GENERAL SUMMARY AND DISCUSSION

The first and most important observation which emerges from the Symposium is that different investigators have had markedly different results. Most workers (Hegnauer, Kay, Swan, etc.) have obtained fibrillation at 26–19° C. but Gollan (who uses a pump-oxygenator) and Lewis (who does not) have cooled animals to much lower levels without trouble. Similar discrepancies are apparent with respect to production of alkalosis-vs.-acidosis. It is doubted that hypothermia, itself, increases the tendency toward fibrillation (Dammann and Muller). A review of the contributions indicates that the entire story can be put together somewhat as follows.

With cooling of the whole body metabolic requirements decrease in all organs, and O_2 use and CO_2 production drop. This decrease in metabolic activity is accompanied by a decrease in cardiac output roughly proportional to the requirement for O_2 transport. There is no evidence that the contractility of the myocardium is impaired (McMillan). If the heart rate is not artificially high or exceptionally low there is no evidence that the cardiovascular dynamics are abnormal (D'Amato). High driven rates, however, will impair filling of the heart, due to prolonged contraction time (D'Amato) and (when O_2 -T begins to equal cycle length) will also influence apparent diastolic thresholds (Brooks), causing a spurious increase. The change in total and absolute refractory periods are those that would be expected from the combined effects of temperature and rate (Brooks, Hegnauer) and thus will vary unless changes in rate are eliminated. With fairly severe cooling, asystole is the

expected end result, and spontaneous rhythmicity will cease at different temperatures in different parts of the myocardium (Brooks, Hoffman). If the body temperature is in the range of 20–10° C. when asystole supervenes, long periods (25+ minutes) of complete lack of perfusion can be tolerated by the heart without any evidence of damage (Gollan, Lewis, others). Furthermore, most studies indicate that, prior to the advent of asystole the cardiac output, in terms of O₂ flow, is at all times more than adequate. Thus, in the simplest situation, there seems to be no problem with respect to hypothermia (Dammann and Muller) as, indeed, is actually the case in studies of isolated cardiac tissues (Hoffman) and as is always seen in hibernators and sometimes in non-hibernators. When difficulties are encountered in hypothermic mammals, they might thus be divided into two major areas: 1) the onset of fibrillation at relatively high temperatures (26°→19° C.) when the body still requires blood flow and 2) the onset of asystole (a normal and expected result) at *too high* a temperature, when flow is still required. If we disregard the requirement for *blood flow*, both these happenings (fibrillation and asystole) provide certain surgical advantages (quiet heart, less danger of air embolism with open heart, etc.).

The problem is thus simplified, at least in superficial analysis, to a search for the factors responsible for onset of fibrillation and asystole at temperatures which are inconveniently high. As a starting point, it is probably easiest to assume that there are multiple factors which may all not always be present and which, in different combinations, can cause trouble. In an analysis of these factors, however, it is immediately apparent that our information is not adequate to permit a full evaluation of each factor.

Inadequate perfusion. Although studies of A-V O₂ differences during hypothermia suggest that O₂ carried to the capillaries is in excess of tissue requirements, we have no conclusive evidence that oxidative metabolism is normal. Thus the rate of oxidative activity may be depressed to an extent that does not keep up with the other metabolic requirements of the tissue. More important, we have little evidence concerning the adequacy of CO₂ removal *from tissues*. Under certain conditions it is possible that serious local accumulations of CO₂ occur in spite of, or even perhaps in part due to the high pO₂.

Also to be considered with respect to perfusion is the question of local changes in electrolyte concentration. Poor perfusion of the extracellular spaces of certain cells will result in (1) local changes in electrolyte concentrations differing from those obtaining elsewhere, and (2) an inability to evaluate local electrolyte shifts from a study of plasma electrolyte levels. Finally data on water shifts between plasma, extracellular and intracellular phases are not adequate.

These findings suggest several areas where added work is required. Information on cellular metabolic activity, local concentrations of CO₂ and ions, and local changes in water distribution are needed. It is possible that accurate studies of pO₂, by means of the so-called oxygen electrode, would show results similar to those obtained on the cerebral cortex—large differences in pO₂ of cells near to and far from capillaries.

The evidence in favor of a perfusion defect is fairly good (Gollan's studies using a pump-oxygenator, and getting no fibrillation, and also his studies of the effect of

coronary occlusion at low temperatures). It is also possible that the effect of *age* of the animal on the response to hypothermia, as well as the reported beneficial effects of *high* pCO_2 in the inspired air may both be related to differences in local perfusion of tissues.

If we consider that, in some instances, tissue perfusion is in certain respects inadequate we are in a position to ask: What are the *direct* results of poor perfusion which might give rise to the undesired effects of hypothermia (i.e., fibrillation and high-temperature asystole)? We should consider the following factors:

1. Anoxia (local).
2. Hypercapnia (local) \rightleftharpoons and pH.
3. Electrolyte imbalances: K^+ ; Na^{++} ; Ca^{++} ; H^+ .
4. Other effects on metabolic activity of cell and cell membrane.
5. Dissimilar effects in various regions or tissues.

Also, the possibility should be considered that at certain critical stages of hypothermia, norepinephrine and/or epinephrine are released (Hume, etc.) or unusual autonomic nerve discharges occur as a part of the nonshivering compensatory mechanism (Keller). Certainly a combination of excitatory action and depression would tend to favor development of arrhythmias (Brooks, *et al.*). Keller's work constitutes a contribution to such studies but it is not as yet sufficiently extensive.

There is ample evidence for the possible role of each, any, and all of these factors from the reports of various investigators.

Hegnauer says that *fibrillators* are identified by S-T segment changes, by low diastolic thresholds and by positive Ca and negative K and H ion balances of myocardium. Taking these statements at face value, what can they imply regarding the underlying mechanisms?

The ECG change noted is entirely non-specific. It can be brought on at unusually high temperatures by giving Ca, and abolished at low temperatures by giving K, PO_4^- , or changing pH; and yet it may persist in spite of pH reversal, and may be brought on at normal temperature by high pCO_2 . It can be caused by local injury or asymmetrical cooling or anoxia, etc.

If this ECG finding has any significance it is that of local differences in the condition of the myocardium with respect to the time-course of repolarization. In the simplest case, it suggests that in some areas the plateau of the action potential has been lost in certain fibers but retained in others. Such a change in the plateau might result from anoxia, ions ($Ca\uparrow$, $K\uparrow$), perhaps from changes in pCO_2 and pH. At any rate, any of these could be an effect of local perfusion deficiency. Not to be ignored, however, is the effect of unequal cooling of the heart, which could give a similar picture if the temperature gradient were great enough.

Studies of isolated heart fibers have *not* revealed significant changes in the critical "threshold" potential level. The next possible cause of a lowering of diastolic threshold is a change in resting potential, and it is interesting that studies of single fibers begin to show a drop in resting potential at the same temperature (around 25–26°) as that associated with a significant decrease in threshold of potential fibrillators.

Again, however, we cannot exclude inadequate perfusion as a contributing factor,

since both local anoxia and local failure to perfuse away K^+ will result in a lowering of resting potential. This hypothesis gains support from the observation that not *all* animals develop the lowering of thresholds; some (possibly those with better perfusion) go on to show a progressive elevation of thresholds and terminal asystole.

The attempt to do balance studies of ions, based on $A-V$ differences and performed in the absence of any study of water shifts is not necessarily meaningful. However, if the *threshold* studies indicate a *decrease* in resting potential, we certainly are entitled to a loss of K^+ from the fiber, and possibly also a loss of H^+ . Our feeling about the positive Ca^{++} balance in particular, and all ion studies in general is that, as long as the methods used cannot explain the finding of a constant negative Na^+ balance in *all* animals under *all* conditions, the other small differences noted are not worth worrying about.

The finding of an increased sensitivity of the heart to Ca^{++} at low temperatures, regardless of pH, is probably accurate. However, this may be related to the finding (see Lepeschkin) that frogs in *winter* have low serum Ca, in summer high Ca, and addition of Ca to winter frogs converts them to summer condition. In other words, we have other evidence that Ca sensitivity of the heart is related to body temperature and perhaps the nonfibrillators, like the frogs, have an adequate mechanism to lower the serum Ca during cooling.

CONCLUDING SUGGESTIONS

It should be stated, without implied criticism of those who have made contribution to this field, that fuller studies are needed. Additional multiple recordings and analyses are required before conclusions can be drawn about many matters discussed in this symposium.

Despite the vast amount of work already done, little is as yet known concerning the action of hypothermia. It is abundantly evident, however, that unless future experiments are well conceived, carefully done, and more extensive, they will add little to our present knowledge.

It is felt that the individual cell and specific reactions must be studied more thoroughly; that the action of hypothermia on membrane function and on the contractile process should be determined; and that a more extensive study than has yet been attempted of the functional changes in the intact heart during hypothermia must be undertaken by groups qualified and equipped to study many aspects of physiology.

PART IV

HYPOTHERMIA IN NEUROSURGERY

E. H. BOTTERELL AND W. M. LOUGHEED

In 1950, Bigelow¹ first suggested the use of hypothermia in cardiac surgery. The purpose of this investigation is to establish the role of hypothermia in neurosurgery, with particular reference to the management of the ruptured intracranial berry aneurysm. Extra-corporeal shunts and local cooling of the brain which were investigated by one of us (W. M. L.) proved to be too difficult for practical use. Experimental studies of general hypothermia² indicated that the cerebral metabolic rate of dogs fell to between 33 per cent and 25 per cent of normal at 25° C. and that these animals could withstand periods of anoxia four times greater than the average survival period at 37° C.

This paper deals with the clinical aspects and evaluation of hypothermia in neurosurgery.

We have operated upon 40 patients using hypothermia. Thirty-two have had berry aneurysms, four arteriovenous malformations, one resection of internal carotid artery, two cerebral tumors, and one hemispherectomy³ (table I).

METHODS

Pre-operative medication consisted of the intramuscular injection of 50 mg. each of Largactil (chlorpromazine), Phenergan and Demerol the night preceding operation, and again in the morning of operation. Lightly anesthetized (N₂O in Trilene) and intubated, the patients were placed in a bath on the operating table (fig. 1). Electroencephalographic, electrocardiographic, and multiple thermocouple leads were connected with the patient. A Courmand needle was placed in the radial artery to measure the mean blood pressure. The neck was dissected with exposure of the carotid and vertebral arteries bilaterally in order to allow occlusion as might be needed (fig. 2). The bath was filled with ice water and the patient cooled. The patients were allowed to breathe spontaneously and were not hyperventilated.

Hypothermia has greatly facilitated and reduced the risk of local surgical treatment of intracranial aneurysms and certain arteriovenous malformations.⁴

The advantages afforded by hypothermia in the surgical management of recently ruptured intracranial aneurysms include reduction of the oxygen demand of the

TABLE I
HYPOTHERMIC CASES

Hemispherectomy	1
Brain tumour	2
Arteriovenous malformations	4
Aneurysms	32
Resection int. carotid.....	1

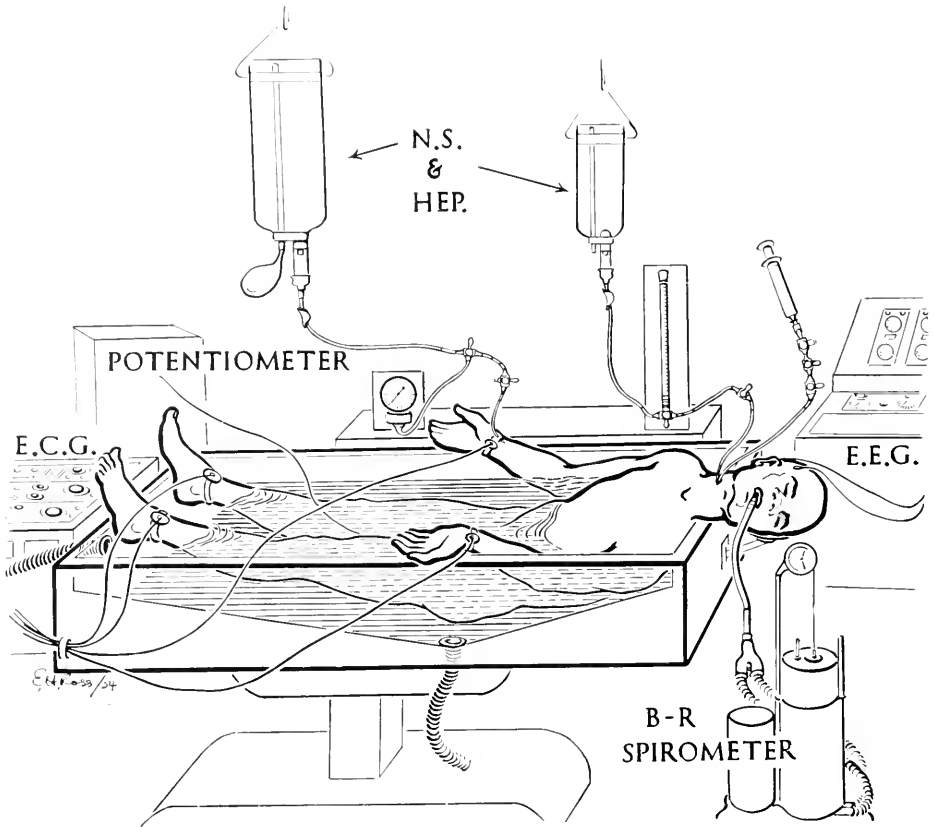


FIG. 1.—Patient in ice-water bath, prepared for operation.

brain and prevention of further cerebral damage from anoxia secondary to spasm of the affected artery. A "slack" brain accompanies hypothermia in a substantial proportion of cases. In a smaller proportion the blood pressure is reduced (fig. 3). Exposure of the aneurysm is therefore made easier with a lessened likelihood of recurrent rupture. Should the aneurysm bleed during exposure, uncontrollable hemorrhage and clipping of the main artery are avoidable, for one may temporarily interrupt the cerebral circulation completely or in part. This is done in safety by occlusion of the carotid or carotid and vertebral arteries in the neck, or the appropriate intracranial artery.

Case report. The case of B.H. demonstrates the advantage of hypothermia. This 45-year-old woman, between 1 July 1955 and 1 August 1955, had four sub-arachnoid hemorrhages. The third and fourth attacks were followed by unconsciousness and confusion, which cleared. She was referred on 29 September to the Toronto General Hospital for further treatment.

Examination on admission revealed the patient to be fully conscious without localizing symptoms save for slight ptosis on the left side.

Arteriograms demonstrated a left supraclinoid aneurysm (fig. 4).

Operation was carried out on 5 October 1955 under hypothermia. At 29.2° C.

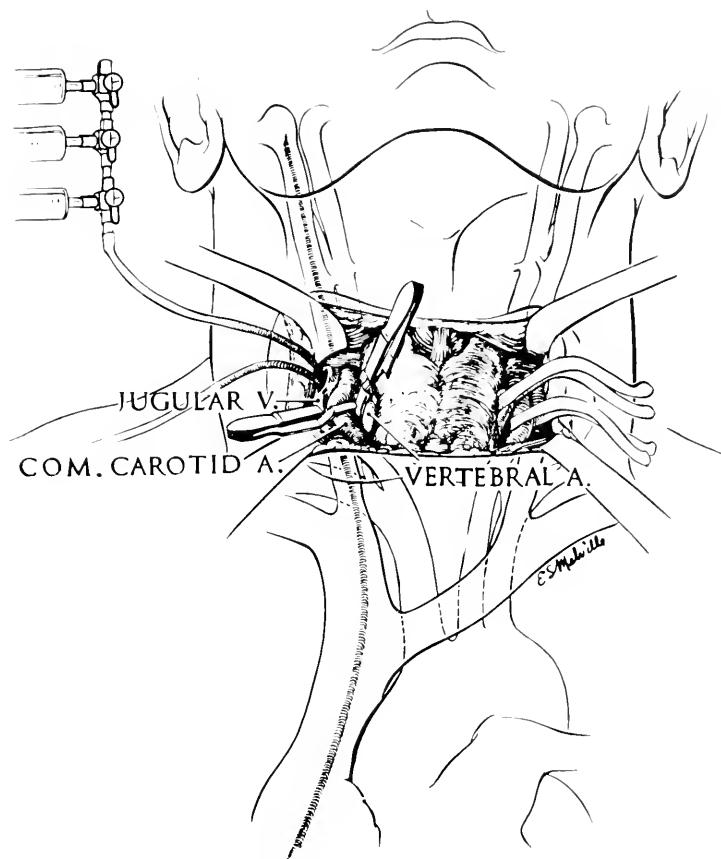


FIG. 2.—Dissection to expose carotid and vertebral arteries.

both common carotid and vertebral arteries were occluded for 10 minutes to reduce bleeding to a trickle. The neck of the aneurysm was clipped and this was reinforced with muscle.

Postoperatively the patient made an excellent recovery, and there was no neurological deficit.

Certain arteriovenous malformations, either because of their situation or large size, or both, have heretofore been impossible to remove. Reduction of blood flow by means of bilateral cervical occlusion of carotid and vertebral arteries can make possible successful surgical removal.

Of the 40 cases, there have been 32 aneurysms of which 18 were operated upon in the acute stage. There have been four deaths among the acute cases (due to overwhelming brain damage), plus one death from ventricular fibrillation. Ventricular fibrillation occurred in a second case, but cardiac resuscitation was successfully performed.

During operation the aneurysm ruptured in 18 instances, and by means of carotid or carotid and vertebral occlusion, a "dry" or "relatively dry" surgical field was obtained, and a clip placed accurately on the neck of the aneurysm (fig. 5). The

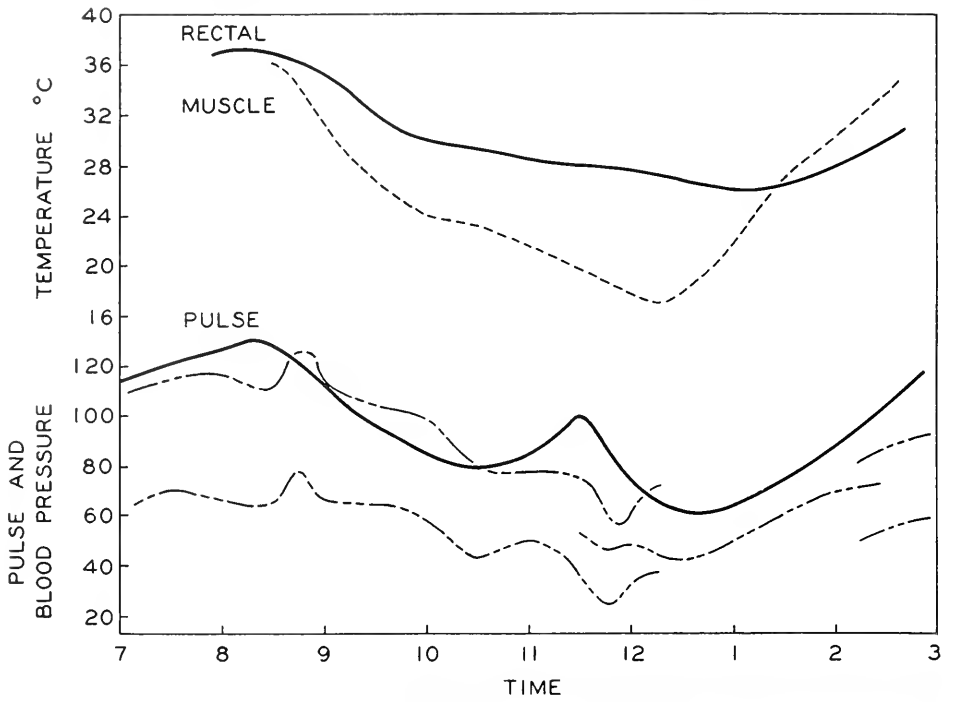


FIG. 3.—Reduction of blood pressure with hypothermia.



FIG. 4.—Arteriogram of patient with left supraclinoid aneurysm.



FIG. 5.—Arteriogram of same patient as in fig. 4, showing clip on neck of aneurysm.

periods of cervical occlusion of both common carotid and both vertebral arteries ranged from two minutes to 13 minutes and 20 seconds, with repeated periods of occlusion in some cases. There has been no clinical evidence of cerebral dysfunction attributable to hypothermia or occlusion of the cerebral circulation save in the case of ventricular fibrillation.

The liabilities inherent in the use of hypothermia, such as ventricular fibrillation, must be weighted against the grave dangers to life from ruptured aneurysms and their treatment. The results have encouraged us to pursue this methodology, but as yet we do not have sufficient experience to warrant definite conclusions. The possibility of operating upon lesions of cerebral blood vessels, supported by interruption of cerebral circulation, may allow development of new and vital reparative operative procedures.

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DISCUSSION

Dr. George Clowes: It is apparent that the recordable electropotentials of the brain disappear below certain temperatures. This "silence of the brain" is also seen after the carotid arteries are occluded at somewhat higher temperatures. It seems to me that the situation is different in each instance. In the first, the brain metabolic and electrical activity probably is almost totally obliterated by cold. In the latter, at a degree of cold normally not obliterating the brain waves, removal of the blood supply causes an event to occur within the brain which stops organized electrical discharge.

It has been shown at normal temperatures that anesthetic agents in sufficient concentrations can depress and ultimately obliterate the brain waves. This is reversible. We have demonstrated that severe hypercapnia uncomplicated by hypoxia has this same effect. However, if an animal's brain waves are obliterated for more than 20 minutes by excess carbon dioxide or anesthetics, it will die in a state resembling normovolemic shock within 12 to 18 hours. Pure hypoxia, on the other hand, produces little effect on the electroencephalogram until the arterial oxygen content has fallen below a critical level of three volumes per cent, after which death rapidly follows. It seems to me that the neurosurgeon who clamps the carotid arteries is dealing with a situation similar to that which I have described at normal temperatures. I should like to ask, how long is it possible to obliterate the cerebral circulation under hypothermia with disappearance of brain waves and still have the animal recover fully?

Dr. Loughheed: I don't think that I can answer the first part of Dr. Clowes' question very well, because when one occludes the cervical vessels to clip the aneurysm, it is a rather tense moment and it is difficult to coordinate the electroencephalographer with the surgeon, so there are gross artefacts in the EEG. We have not noticed that the EEG right after this period of occlusion has been disturbed. The portions of EEG that are not disturbed by artefact during the occlusion were normal.

Animals breathing 100 per cent nitrogen at a temperature of 25° C. have not shown any change in their brain waves for a period of 15 to 22 minutes. I have never taken an animal or a patient to the stage of electro-silence, so I can't answer that part of it.

Dr. Botterell: This work was done primarily as a clinical effort to improve our success, or lack of it, in dealing with these ruptured aneurysms.

One can expand a bit on what Dr. Loughheed said about the EEG, for there are surprisingly few changes in ten minutes of bilateral carotid and vertebral artery occlusion. There is some lowering of the amplitude and disappearance of fast activity, but nothing in the way of spikes or high voltage slow waves. Dr John Scott has been studying these records. We have the needle electrodes in the scalp and a constant recording throughout the operation and cooling.

It might interest you to know that the pH of these patients has ranged from 7.4 to 7.45 in the last dozen cases. We have been able only to make these physiological observations in the course of our clinical treatment, so they are perhaps not as full as some of the experimental work.

EFFECT OF HYPOTHERMIA ON TOLERANCE TO HEMORRHAGIC SHOCK*

E. W. FRIEDMAN,† D. DAVIDOFF AND J. FINE

With the technical assistance of Dorothy Kaufman

During peripheral vascular collapse the disparity between the tissue requirements for blood and the amount available results in rapid impairment of many vital functions. Since hypothermia reduces this imbalance by reducing the tissue requirements, its value as a means for the preservation of tissue function during peripheral vascular collapse deserves exploration. A beneficent effect upon hemorrhagic and tourniquet shock by lowering the environmental or body temperature has been observed. Thus, Warren¹ noted a lower mortality rate in tourniquet shock at a room temperature of 16° C. as compared to a room temperature of 28° C., and Delorme² and Adams-Ray³ state that the hypothermia protects the dog from otherwise lethal severe hemorrhagic shock. The studies described below deal with the effect of hypothermia on hemorrhagic shock in the dog with particular reference to the cardiovascular dynamics and survival rate.

PRELIMINARY STUDIES WITH VENO-VEINUS COOLING

Various methods of cooling were considered in order to find the one that would best satisfy the requirements of our standard hemorrhagic shock experiment.⁴ Since the development of a state refractory to transfusion is a function of time, we sought the method which would produce the desired reduction in body temperature as rapidly as possible. Veno-venous cooling⁵ has the disadvantage that an extracorporeal circuit imposes upon the circulation. But the promise of very rapid cooling (1° every 4 minutes) with a minimum of shivering and a low incidence of ventricular fibrillation led us to try it.

The cooling equipment consisted of a rotary pump which sucked blood from the superior vena cava and drove it back into a femoral vein after circulation through a coil immersed in an ice bath. All tubing except for a rubber segment in the pump was polyvinyl plastic, 15 feet in length, connected to plastic cannulus inserted into the superior vena cava *via* the jugular vein, and into the femoral vein. The femoral artery was cannulated for sampling, pressure readings, and bleeding. The trachea was cannulated for measurements of respiratory function and oxygen consumption. Mixed venous blood for measurement of cardiac output (Fick method) was obtained from the caval cannula advanced into the right auricle. Because of the desirability of avoiding barbiturates and anesthetics in the animal to be put into shock, the foregoing procedures were performed under local anesthesia.

All dogs were given 2mg./kg. of morphine one to two hours before the experiment. After 2 mg./kg. of heparin was injected intravenously, the extracorporeal

* Supported by a contract with the Research and Development Division, Office of the Surgeon General, U.S. Army; and by a grant from the U.S. Public Health Service.

† Fellow of the American Heart Association.

circuit was filled with 150 ml. of donor blood. The pump was then started and its speed regulated so as to return 100, 200, or 300 ml. of blood per minute.

Cooling in normal dogs. At a rate of 300 ml./min. through the pump the temperature of the returning blood ranged from 22° C. to 10° C. Shivering began as soon as the pump was started, and continued with increasing severity until convulsive seizures occurred, or narcosis from cold supervened. Because of the shivering, the average time required to reduce the rectal temperature of 28° C. averaged two and one-half hours. We could not safely lower the rectal temperature below 28° C. because ventricular fibrillation occurred without warning in three of seven dogs (at 26° C. in two and at 27° C. in one).

When the rectal temperature reached 28° C., circulation through the pump was stopped. To keep the rectal temperature at this level it was sufficient to operate the pump for five minutes every half-hour at a rate of 100 ml./min. Cessation of pumping was followed by a spontaneous rise in temperature of 2° C. per hour, and circulating the blood through the coil immersed in water at 40° C. raised the rectal temperature to 34° C. in about one hour. Of four dogs which were rewarmed after hypothermia of six hours duration, two survived indefinitely and two died, one in 12 hours and one in 60 hours, both with pulmonary atelectasis.

The data in table I show that in the unanesthetized dog a rectal temperature of 28° C. substantially reduces arterial pressure, respiratory rate, pulse rate and cardiac output and increases the oxygen consumption and the A-V oxygen difference. Although the volume of air breathed per minute per 100 ml. of oxygen consumed ("ventilation equivalent") is reduced, the arterial oxygen content remains normal.

Cooling in hypovolemic dogs. Dogs were bled into an elevated reservoir from the femoral artery until the arterial pressure fell to 30 mm. Hg. This level was reached about five minutes after bleeding was started. Cooling was begun at this time and a rectal temperature of 28° C. was reached within an hour. Starting the pump induced an abrupt return of about 100 ml. of blood from the reservoir to the dog. On stopping the pump this blood returned to the reservoir. Shivering did not occur. The dogs were quiet and weakly responsive to ordinary stimuli.

Of a total of six experiments, four were not completed because of technical difficulties in two and ventricular fibrillation in two others (in one at 34° C. and in another at 32° C.). In the two completed experiments the hypotensive period was

TABLE I

CARDIOVASCULAR AND RESPIRATORY DYNAMICS IN SEVEN UNANESTHETIZED DOGS PRIOR TO AND DURING HYPOTHERMIA (28° C.) BY THE VENO-VENOUS COOLING METHOD OF ROSS^a

Body temperature	Arterial pressure (mm. Hg)	Pulse rate (min.)	Cardiac output (ml./min.)	Arterial O ₂ (vol. %)	A-V O ₂ diff. (vol. %)	Pulmonary ventilation (l./min.)	Oxygen consumption (ml./min.)	V _{ent. equiv.} (l./min./100 ml. O ₂ consumed)	Respiration rate (min.)
38-39° C.	131	149	2740	18.2	3.8	15.2	85.4	16	51
28° C.	85	97	1600	19.1	8.5	13.4	134.0	8.6	25

^a The data are averages.

5 hours in one and 4½ in the other, during which time about 40 per cent of the shed blood had returned to the animals. The rest was then transfused. The temperature rose along with the rise in blood pressure, but the pressor response was inadequate and not sustained. Both dogs were dead several hours later. The postmortem findings in the two completed experiments were characteristic of irreversible hemorrhagic shock.

The hemodynamic data of both experiments are similar to, and are included in, table IV below with the data from experiments with external cooling.

Because the time required to lower the temperature in the unanesthetized dog in shock was longer than we considered desirable for our purposes, because of the unpredictable occurrence of ventricular fibrillation during shock as well as before, and because of the undesirable additional load of an extracorporeal circuit, we abandoned the veno-venous cooling method.[‡]

COOLING BY IMMERSION IN ICE WATER

This is a much simpler technique than direct cooling of blood and was used in all subsequent experiments. We preferred ether rather than barbiturates as an anesthetic for precooling because, by allowing a short interval for desaturation before shock is induced, virtually all of it is eliminated. Shock dispenses with the need not only for further anesthesia, but also for further cooling because the body temperature continues to fall and remains below 28° C. until a transfusion is given.

Experimental procedure. Mongrel dogs weighing 15–25 kg. received morphine sulfate (2 mg./kg.) and were lightly anesthetized with ether to prevent shivering. After immersion in ice water for one hour the rectal temperature fell to 28° C. The anesthesia was then stopped, heparin (2 mg./kg.) was injected intravenously, and the animal was removed from the ice water. Time for desaturation of ether was allowed. The dogs were then bled from a cannulated femoral artery into a bottle elevated so as to maintain the arterial pressure at 30 mm. Hg. as previously described.⁴ The rectal temperature continued to drop during the next half hour, and generally leveled off at about 23° C., where it remained for two hours. (In two of fifteen experiments the rectal temperature dropped to 19° C.) It then began to rise one-half degree every hour so that at the time of transfusion it ranged between 25–28° C.

The amount of blood entering the elevated reservoir reached a maximum in 30 minutes, and remained at that level from one to two hours, after which blood returned to the dog at about 25 ml. per hour, which is very much slower than in uncooled dogs. After 40 per cent of the maximal bleeding volume had spontaneously returned to the animal, or after an arbitrary period of eight hours of hypotension, whichever occurred first, the femoral artery was clamped and the blood remaining

[‡] Precooling by any method requires anesthesia to prevent shivering. Cooling started during shock does not produce shivering and does not require anesthesia. In the definitive experiments with external cooling described below, we were obliged to use ether for cooling prior to inducing shock. Our experience does not permit a judgment as to the relative merits of the Ross method and external cooling when anesthesia is employed for precooling. But for cooling initiated during shock external cooling proved superior to direct cooling of blood in that ventricular fibrillation, within the temperature limits employed, did not occur and was in general a less hazardous burden on the animal.

in the reservoir was warmed to 37° C. and reinfused *via* the femoral vein. Because rapid transfusion precipitated ventricular fibrillation in the first two experiments, the rate thereafter was restricted to 5 ml./min. At this rate, and with constant observation of the pulse and immediate cessation of transfusion on the first sign of cardiac irregularity, ventricular fibrillation during transfusion was avoided. When the arterial pressure had risen to 60 mm. Hg, the animal was immersed in a water bath (40–45° C.) to hasten warming. After completion of the transfusion the dogs were given 25 grams of glucose intravenously to counteract the development of hypoglycemia. The blood pressure continued to rise concurrent with the rise in rectal temperature until normal limits were restored. When the animals were removed from the bath and dried, some struggling usually occurred. Occasionally weakness or paralysis of the hind legs, especially of the leg with the cannulated femoral artery, occurred and lasted about two days.

Adequate spontaneous respiration persisted at all temperatures as low as 19° C., so that artificial respiration was not required.

Data were obtained on pulmonary ventilation, oxygen consumption, cardiac output (Fick method), A-V oxygen difference and blood pH (Beckman glass electrode pH meter).

Three types of experiments were performed. Group I (14 dogs) were treated as described above. Group II (9 dogs) received in addition one million units of crystalline potassium penicillin-G intravenously and 0.5 gram streptomycin intramuscularly at the time of transfusion, and twice daily for three days thereafter. From the therapeutic point of view, it was necessary to determine the importance of the time of application of the hypothermia. Accordingly, in Group III (10 dogs) the cooling was started during shock when the level of hypotension was reached. The rectal temperature fell to 28° C. within one hour. In these dogs antibiotic therapy was given as in Group II.

All dogs which died or were killed were submitted to gross postmortem examination.

RESULTS

Effect of hypothermia on tolerance to hypotension and on survival rate in hemorrhagic shock (table II). Previous experiments on uncooled dogs, which received no antibiotic or antibiotics as given in these experiments, provide the control data.⁶ These show that after about five hours of hypotension (30 mm. Hg) 40 per cent of the shed blood will have returned from the reservoir to the circulation. At this time the rate at which the blood is returning spontaneously is insufficient to sustain the selected level of hypotension, so that the blood remaining in the reservoir must be rapidly transfused to sustain the blood pressure. The resulting immediate and substantial pressor response is transient, and death follows after an average of six hours of shock. The survival rate is some 15 per cent or less. Survival in these experiments means survival in good condition for at least five days after transfusion.

In all three groups of experiments with hypothermia the capacity to tolerate severe hypotension is distinctly greater than in uncooled animals. The volume of blood returning to the dog from the elevated reservoir within the period of observa-

lethal factor was involved. The postmortem examinations disclosed none of the normal stigmata of shock, or evidence sufficient to implicate hypothermia as the cause of death, for nothing more than an occasional instance of pulmonary atelectasis was found. Since we had already demonstrated that traumatic shock in normothermic animals severely impairs the antibacterial defense, and that this impairment persists for at least 48 hours after transfusion in surviving animals, we considered the possibility that infection might account for the late death in cooled animals, in spite of the absence of the customary evidence of infection. In the subsequent series of experiments (Group II, table II) this was investigated by treating the dogs in precisely the same manner as the dogs in Group I except that, as stated, antibiotic was administered at the time of transfusion and twice daily for the three subsequent days. The result was 100 per cent permanent survival!

In uncooled dogs antibiotics given at the time of transfusion proved useless, in contrast to the very considerable protection they afforded when given prior to shock.⁶ The explanation for the difference in the result due to timing of the antibiotic is that loss of resistance to bacteria develops rapidly during the shock state so that some bacterial toxin is produced in the absence of antibiotic. At the same time the animal becomes rapidly and increasingly vulnerable to toxin so that if only a tiny fraction of the dose that is lethal to the normal animal is produced it may become a fatal dose as the shock continues, since it can meanwhile be neither detoxified nor excreted.⁷ The development of irreversibility to transfusion appears, therefore, to be related to the amount of toxin to which the animal is exposed before an antibiotic to halt further toxin production is administered. Since antibiotic therapy at the time of transfusion is ordinarily useless, but is the sole agent responsible for survival in the precooled animal, the conclusion follows that the protection which precooling confers upon the animal in shock consists in shielding the antibacterial defense mechanism. That this is true is further demonstrated in experiments to be reported elsewhere,⁸ the results of which were briefly as follows: In uncooled dogs transfusion after two hours of shock nearly always results in permanent recovery. Nevertheless, the antibacterial defense has been damaged because such dogs uniformly succumb to an intravenous dose of bacteria which the normal dog can readily dispose of.⁹ This dose of bacteria is fatal whether given during the shock period or anytime within 24 hours after transfusion. On the other hand, if the dogs are precooled, the bacteria are readily eliminated, and the dogs survive.

In the group of experiments in which cooling was started *after* shock was induced (Group III), and with antibiotic given as in the Group II experiments, the protection achieved was far less than by precooling, i.e. a survival rate of 30 per cent in Group III as contrasted to 100 per cent in Group II. From the evidence given this is because during the interval between the induction of shock and the delayed application of hypothermia some damage to the antibacterial defense mechanism in consequence of deficient peripheral flow occurs and some bacterial toxin is produced. Precooling is superior to delayed cooling because it not only minimizes the damage to the defense mechanism during this interval, but probably also because it at the same time minimizes or totally prevents the production of bacterial toxins. In this latter respect prophylactic hypothermia achieves the same

TABLE IV

CARDIOVASCULAR AND RESPIRATORY DYNAMICS IN DOGS COOLED AFTER INDUCTION OF HEMORRHAGIC SHOCK

	Rectal temp. (°C.)	Arterial pressure (mm. Hg.)	Pulse rate (min.)	Cardiac output (ml./min.)	Arterial O ₂ (vol. %)	A-V O ₂ diff. (vol. %)	Pulmonary ventilation (L./min.)	O ₂ consumption (ml./min.)	Ventil. equiv. (L./min./100 ml. O ₂ consumed)	Respiration rate (min.)
Prior to cooling or shock	38-39	136	150	2920	19.2	6.3	19.6	164.4	12	34
During shock	38-39	30	166	724	17.0	13.2	18.6	99.5	18	32
During hypothermia and shock	24	30	84	366	17.6	14.2	15.5	68.8	30	21
After transfusion and rise in body temperature	33.2	106	90	4572	19.1	4.2	22.7	193.4	13.8	21
Number of experiments for each value listed	(10)	(10)	(10)	(4)	(4)	(4)	(4)	(4)	(4)	(10)

kind of protection as prophylactic antibiotic therapy, though by a different action upon the offending bacteria.

The effect of hypothermia on the cardiovascular dynamics of hemorrhagic shock (tables III and IV.) In the unanesthetized dog hypothermia (28° C.) lowers the blood pressure, pulse rate and cardiac output, but the pulmonary ventilation is adequate to sustain the oxygen content of the arterial blood. The considerable increase in oxygen consumption and in the A-V oxygen difference are due to muscular activity, for when shivering is prevented by ether the oxygen consumption is considerably reduced rather than increased, and the A-V oxygen difference is unchanged. Hence the rise in the A-V difference in the unanesthetized dogs appears to result from the increased extraction of oxygen by muscle.

After the induction of shock the pulse rate continues to fall instead of rising as it does in the normothermic dog. The precipitous further decline in cardiac output is such as to reduce the stroke volume to less than 3 ml. With an oxygen consumption only 20 per cent of normal during shock, the sharp rise in the A-V oxygen difference must signify an almost static peripheral circulation. For this reason, together with the feeble effect upon caval flow of the much reduced respiration, return flow to the heart is extremely small. The resultant slow flow through the pulmonary circuit allows the very low ventilatory exchange to provide enough oxygen to maintain an adequate arterial oxygen content. Because of the low level of tissue metabolism, hypercapnia does not develop in the face of a depressed respiration, as indicated by the fact that no significant shift in blood pH occurred in our experiments (table V). Therefore, we were not obliged to employ hyperventilation.[§]

Following transfusion and warming there is a return to normal or nearly normal values in all categories. The notable increase above normal in oxygen consumption

[§]The death of hypothermic dogs in shock cannot be explained as due to the effects of hypothermia upon pulmonary efficiency because such a death would not be prevented by an antibiotic, unless it could be shown that the primary effect of deficient ventilation is pulmonary infection.

TABLE V

BLOOD pH IN NINE DOGS PRIOR TO AND DURING HEMORRHAGIC SHOCK PLUS HYPOTHERMIA (24° C.)^a

Dog no.	Control	During shock plus hypothermia
1	7.20	7.20
2	7.20	7.18
3	7.37	7.30
4	7.40	7.40
5	7.45	7.45
6	7.4	7.4
7	7.44	7.48
8	7.28	7.40 ^b
9	7.52	7.60 ^b

^a Produced by surface cooling.

^b Shock induced prior to cooling.

may be due to resumption of greater muscular activity than occurred during the control period under morphine.

Comparison of the foregoing data with those from dogs which are cooled after the induction of shock reveals several points of interest. The fall in oxygen consumption and cardiac output is appreciably less than in the precooled dog, and the respiratory depression is not nearly so steep. The higher level of metabolic activity, as reflected in the higher oxygen consumption, suggests that when the circulation is already defective external cooling may fail to reduce the temperature of some of the deeper tissues to the same level as in the precooled animal.

That hypothermia reduces bleeding volume is evident from the data in table II. One cannot attribute the greater tolerance to shock in precooled dogs (Group I and II) to this effect of hypothermia because dogs bled prior to cooling (Group III) suffered a greater than average blood loss and also showed a greater tolerance to shock. Since the antibiotic therapy alone cannot account for the difference in survival rates in these three groups of dogs, we conclude that some property of hypothermia other than its effect of bleeding volume must account for its influence upon the course of the shock state.

COMMENTS

Deterling *et al.*¹⁰ observed ventricular fibrillation in 40 per cent of 16 normal dogs under barbiturate anesthesia at temperatures below 23° C. Spurr *et al.*¹¹ observed this complication in 33 per cent of normal dogs cooled to 20–26° C. for four hours. In a series of 34 shocked dogs at temperatures ranging from 22–28° C., we encountered fibrillation in only 6 per cent. Bigelow *et al.*¹² lowered the incidence of fibrillation by a 50 per cent reduction in blood volume, and attributed the cause of fibrillation to an increase in venous pressure. Our own observations are consistent with this view, for we found that rapid transfusion *via* the femoral vein is well tolerated by normothermic dogs in hemorrhagic shock, but precipitates ventricular fibrillation in hypothermic shocked dogs. The lowered irritability of cardiac muscle during shock may contribute to the lower incidence of fibrillation in our series of shocked dogs.

The acidosis of hypothermia resulting from hypercapnia¹³ apparently does not occur in shocked dogs. This is doubtless because the level of metabolic activity is so low that the correspondingly low ventilatory volume is sufficient to discharge the CO₂ produced. That the death of hypothermic shocked dogs which did not receive antibiotics at the time of transfusion was not related to hypercapnia is obvious from the fact that death was regularly prevented by antibiotic therapy.

Fisher *et al.*¹⁴ observed hypoglycemia and prolonged clotting time as a result of hypothermia of 5 to 10 hours duration. Firor¹⁵ observed altered cell morphology in tissue cultures grown at 28° C. Within the limits and duration of hypothermia which we employed these and other deleterious effects of hypothermia are not dominant features in the shocked dog. On the one hand, the protective action of hypothermia is impressively demonstrated by (1), the experiments of Allen,¹⁶ who found that hypothermic rats survive a dose of total body X-radiation which is fatal to normothermic rats, (2), by experiments showing that hypothermia prevents the swelling of the hind limbs following removal of tourniquets applied for 4–5 hours, and (3), the observation that hypothermia protects the abdominal viscera from an otherwise fatal exposure to total ischemia.^{2, 3, 17, 18, 19} Similarly, we have observed that hypothermia reduces the volume of peritoneal exudate in experimental fecal peritonitis in dogs.²⁰ This may be due to decreased capillary permeability or to vasoconstriction. Presumably the relatively bloodless state of surgical wounds during hypothermia is due to slow flow through the peripheral vessels because of vasoconstriction, lowered blood pressure and reduced cardiac output. Additional beneficial effects of hypothermia, as our observations on hemorrhagic shock demonstrate, are the suppression of bacterial activity, and the support given to the maintenance of the integrity of the antibacterial defense mechanism.⁸ All of these positive qualities of hypothermia are derived from its capacity to reduce the tissue requirements for oxygen and blood supply. When applied within certain limits of degree and duration for certain specific objectives the protective properties of hypothermia outweigh its dangers.

SUMMARY AND CONCLUSIONS

(1) The dog's capacity to tolerate severe hemorrhagic shock is substantially increased by precooling to 28° C. Used alone it does not effect survival, but when combined with antibiotic therapy, it results in 100 per cent survival.

(2) The increased resistance to hemorrhagic shock is due to the protection of the antibacterial defense mechanism, which disintegrates rapidly in the normothermic dog.

(3) The very low level of respiratory activity does not result in hypercapnia, or deficiency in oxygen supply because of the extreme reduction in the rate of metabolic activity. This accounts for the fact that survival is possible even though the cardiac output and peripheral flow are at levels far below those consistent with survival of normothermic dogs.

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DISCUSSION

Dr. E. Frank: I would like to show you some extended studies based on continuation of Dr. Friedman's work in an effort to define some of these antibacterial protective mechanisms as provided by hypothermia in hemorrhagic shock.

Table I shows five groups of experiments. If a single group of dogs is given a standard dose of bacteria, in these experiments *E. coli*, they tolerate it quite well (Group I). If a separate group of dogs, Group II, is subjected to two hours of hemorrhagic hypotension, then retransfused, they, too, can be expected to live. If, however, you combine those two procedures, namely two hours of hemorrhagic hypotension plus intravenous bacteria, you wind up with 100 per cent mortality within one to three days (Group III). In other words, the two hours of hypotension, though not lethal in itself, has done something to make it more possible for bacteria to go on and kill that animal.

However, in Groups IV and V you will notice that if the dog is cooled prior to

TABLE I

EFFECT OF HYPOTHERMIA UPON RESISTANCE TO BACTERIA GIVEN DURING OR AFTER SHOCK OF TWO HOURS' DURATION^a

Group	Experiment	No. of dogs	Survivors	
			Number	%
I.....	E. coli intravenously in normal dogs.....	10	10	100
II.....	2-hour shock in normothermic dogs.....	10	—	90
III.....	2-hour shock in normothermic dogs, E. coli intravenously during shock or at any time up to 24 hours after transfusion.....	30	0	0
IV.....	Hypothermia (28 C.) induced prior to 2-hour shock, E. coli intravenously during hypotensive period—warmed to normal temperature after transfusion.....	8	6	75
V.....	Same as Group IV except E. coli given after body temperature was back to normal ^b	14	11	79

^a Hemorrhagic shock—bleeding to blood pressure 30 mm. Hg for 2 hours followed by transfusion of all shed blood.

^b The results for E. coli—four of these experiments were done with a coagulase-positive hemolytic staphylococcus aureus, to which normal and 2-hour shock dogs respond as they do to E. coli.

the two-hour shock period, with intravenous bacteria given either during hypothermia and hypotension (Group IV) or after rewarming (Group V), the result is that about three-quarters of the animals survive.

The nature of this protection we cannot begin to state, although we may have some inkling of a possible mechanism as a result of our recent cooperation with Dr. Pillemer of Western Reserve. Dr. Pillemer has recently described a substance in serum which he called properdin and which he believes, and we believe with him, may play an important part in natural resistance to bacteria.

Figure 1 shows a characteristic response of properdin titer in a dog subjected to

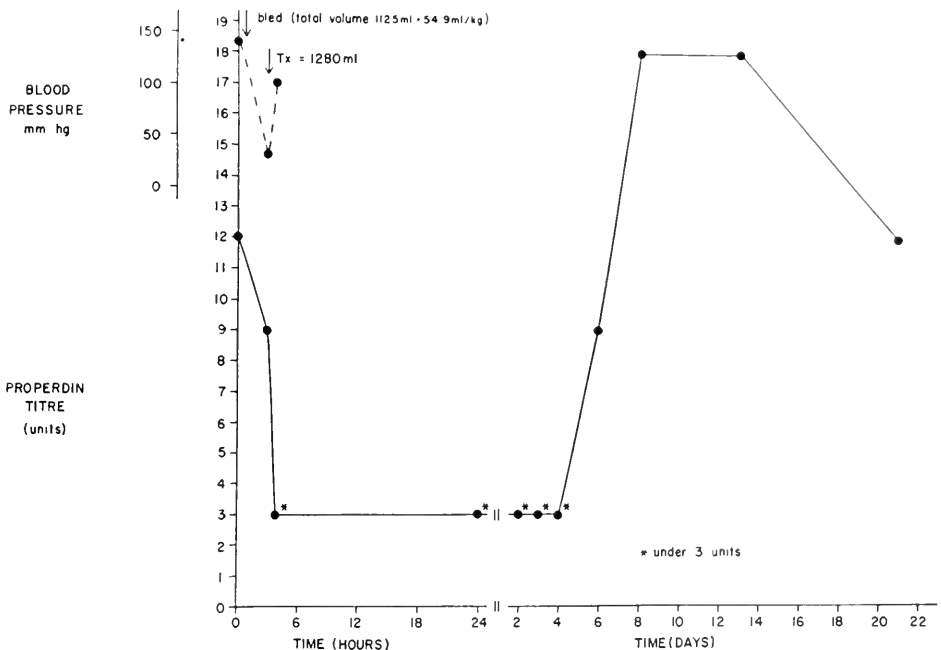


FIG. 1.—Response of properdin titer in dog subjected to hemorrhagic hypotension.

TABLE II

PROPERDIN TITERS OF DOGS SUBJECTED TO HEMORRHAGIC SHOCK DURING HYPOTHERMIA

Dog no.	Body temp.	Bleeding volume (cc./kg.)	Arterial pressure (mm. Hg)		Properdin titers during shock		
			Mean	Range	Initial	2 hrs.	6 hrs.
P-52.....	Under 28° C.	37	45	30-60	12	12	12
P-53.....	Under 28° C.	39	40	30-60	12	12	12

a brief period of hemorrhagic hypotension from which he survives. The properdin titer falls markedly within the first two or three hours. It remains low for several days, and then overshoots. However, when two animals were subjected to hypothermia while their blood pressure was being lowered, the properdin titers remained high not only for two hours but for six hours as well (table II).

We are intrigued by these findings and intend to pursue this matter in the near future.

EXPERIMENTAL OBSERVATIONS ON THE INFLUENCE OF HYPOTHERMIA AND AUTONOMIC BLOCKING AGENTS ON HEMORRHAGIC SHOCK *

ROBERT C. OVERTON AND MICHAEL E. DE BAKEY

As a result of the pioneer work of Bigelow,⁸⁻¹¹ the efficacy of general body hypothermia in obviating the ischemic consequences of acute circulatory interruption has been widely documented.^{2, 13, 18, 19, 22, 24, 25, 47, 50, 51, 51, 55, 61, 64, 67, 68} More recently the concept has been advanced of the state of "artificial hibernation," mediated by the combination of hypothermia and certain autonomic drugs, in which the organism is believed to be resistant not only to acute total ischemia, but also to the wide range of noxious stimuli which lead to traumatic and hemorrhagic shock.^{6, 7, 14, 16, 17, 23, 26, 37, 38, 40, 41, 43-46, 49, 66, 70} Broad claims have been made, particularly by French investigators, for this concept and its clinical applications, extended even to its administration to battle casualties in Vietnam.^{15, 39, 46, 52} Their method involves use of body cooling together with a "lytic cocktail" of certain drugs, of which chlorpromazine seems to be the most important.²⁷ Highly significant as the French work may be, the picture is obscured by the polytherapy employed. It seemed important, therefore, to investigate the problem by a carefully controlled study directed toward ascertaining the significance of the various factors involved in the French protocol.

Because of considerable experience in the study of "irreversible" hemorrhagic shock by the method of Fine,^{30, 35} it was considered desirable to employ this shock preparation and to investigate the effects of hypothermia alone, chlorpromazine alone, and a combination of these two factors on the course of the shock experiment.

Method. The procedure employed in the production of shock was essentially similar to that previously described.³⁵ Healthy, afebrile mongrel dogs, averaging 14.5 kg. in weight, were bled via the femoral artery into an elevated reservoir adjusted at such a height that the blood pressure equilibrates at 30 mm. of mercury. The bleeding volume reached a maximum usually in about one hour; thereafter, as the animal's compensation failed, blood was gradually and spontaneously "taken back" in order to maintain the blood pressure at 30 mm. of mercury. After 40 per cent of the maximum volume had thus been taken back, the remainder was rapidly retransfused. If this "40 per cent end-point" was not reached in eight hours, the experiment was arbitrarily terminated by retransfusion. Although after retransfusion the animals appeared temporarily improved, in our experiments the mortality from this shock preparation has averaged over 90 per cent. In addition to the survival rate, the dog's response to the experiment and to any therapy employed was gauged by the maximum bleeding volume in cubic centimeters per kilogram and also by the duration of hypotension necessary to attain the end-point.

In both Fine's original work and our previous studies, a local anesthetic was used. In the present study, because of the hypothermia and the necessity for obviating

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shivering, general anesthesia induced with morphine sulphate (10 mg./kg.) and pentobarbital (15 mg./kg.) intravenously was employed. It was realized that this might increase the stress of the procedure, but the factor was kept constant by its application to all animals, including the control dogs. The tracheas were intubated perorally, but insufflation was not employed. The anesthetized animals were wrapped in rubberized blankets through which refrigerated fluid was circulated until their temperature reached 31° C. Parallel controls were treated identically except for the cooling. Chlorpromazine was given in dosages of 50 and 100 mg. intramuscularly one to two hours before bleeding to one group and in 5 mg. doses to another. The difference in 50 mg. and 100 mg. dosage has no statistical significance, both being large doses for the dog. Parallel controls were treated identically except they were not given the drug. In the series receiving combined therapy, chlorpromazine was administered in dosage of 50 mg. approximately one hour before initiation of cooling, and on the average approximately two hours before the beginning of the bleeding to one group, and in 5 mg. doses to another.

The experiment was performed on 46 dogs with hypothermia alone, 45 dogs with 50 mg. of chlorpromazine, 20 dogs with 5 mg. of chlorpromazine, 38 dogs with combined chlorpromazine (50 mg.) and hypothermia, 14 dogs with combined chlorpromazine (5 mg.) and hypothermia, and 50 control dogs. Slight (1–2° C.) cooling was secured by the autonomic drug alone, and cooling was achieved somewhat faster in the dogs receiving the drug before the application of surface cooling. The discrepancy in number of controls is due to the simultaneous use of one control to parallel one or more of the other groups.

Results. Originally the survival period was arbitrarily determined at the end of 24 hours, since the majority of deaths occurred well within this time. Most of the controls expired at or soon after the end-point. Subsequently it seemed desirable to determine ultimate survival, based upon a minimum period of 2 weeks. Accordingly the experiments were repeated on additional groups of animals for this purpose. Certain studies carried out in the first groups were also repeated in the second groups thus providing additional data.

Both the immediate as well as the ultimate survival rates were increased in all of the experiments as compared with the control animals (figs. 1 and 2). As might be expected the ultimate survival rates were somewhat less, except for one group of animals, than the immediate survival rates, since in the former groups a number of variables are introduced, such as bacterial contamination and lack of supportive measure. In this connection it has been observed in another experiment conducted in our laboratory that the combined use of anesthesia and hypothermia alone is followed by a mortality of 10 per cent when anti-bacterial therapy is not employed.²⁰

The high proportion of rapid deaths in the control dogs, which is slightly greater than that obtained in our previous studies, testifies to the severity of the stress. Although there was a significant increase in survival rate among the dogs that were cooled before being bled, and an even greater increase in those receiving chlorpromazine in both large and small doses prior to the experiment, the dogs treated by the combined use of hypothermia and chlorpromazine in large doses showed the most pronounced increase in survival. The combined use of hypothermia and small doses of chlorpromazine resulted in a survival rate greater than that following use of the drug alone in comparable dosage, but slightly less than when used alone in

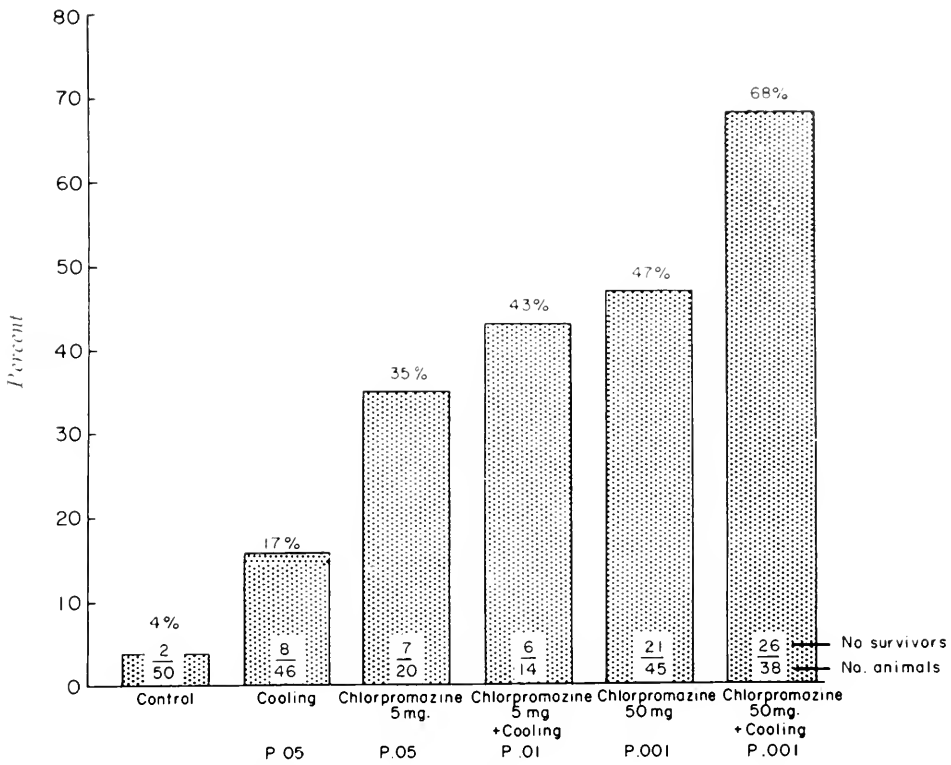


FIG. 1.—Immediate survival in experimental hemorrhagic shock.

larger doses. These results are valid statistically at P values of $<.05$ significance level for cooling alone and for 5 mg. of chlorpromazine alone, $<.01$ for combined cooling and 5 mg. dosage of chlorpromazine, and $<.001$ for 50 mg. dosage of chlorpromazine either alone or combined with cooling.

A protective mechanism is also suggested by the increased tolerance to duration of hypotension, or duration of shock, before the 40 per cent end-point was reached. Whereas in only 8 per cent of the control animals was the blood pressure maintained at 30 mm. Hg for eight hours without having to retake 40 per cent of their maximum bleeding volume, among the treated animals this figure was significantly greater. Particularly striking in this connection is the high proportion of "tolerance" hypotension among those treated by the combined use of hypothermia and chlorpromazine (fig. 3). At arbitrary completion at eight hours the control animals were 61.8 per cent short of the 40 per cent end-point, whereas the hypothermic dogs were 40.3 per cent, the 5 mg. chlorpromazine-treated 77 per cent, the 50 mg. chlorpromazine treated 79 per cent, the cooled and 5 mg. chlorpromazine-treated 72 per cent, and the cooled and 50 mg. chlorpromazine-treated were 80.3 per cent. This may be considered a further reflection of compensation to hypotension without necessity for replacement.

Additional support of tolerance is manifested by the average duration of hypotension, including the animals that were arbitrarily terminated at eight hours. The

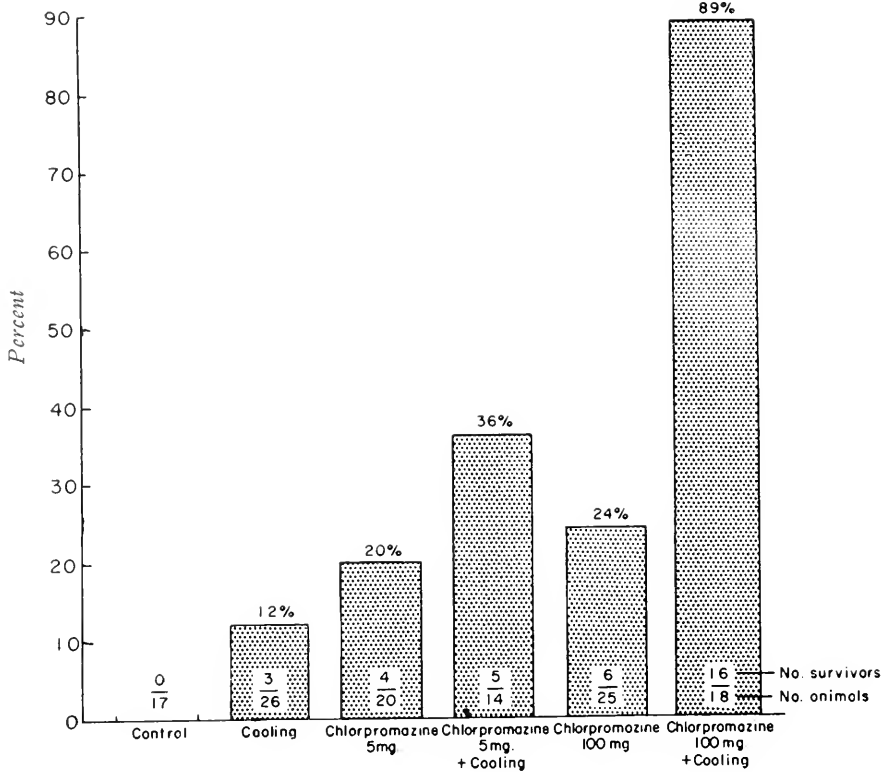


FIG. 2.—Ultimate survival in experimental hemorrhagic shock.

average period of hypotension for the control dogs was 3 hours, 54 minutes, for the hypothermic animals 5 hours; for the 5 mg. chlorpromazine-treated 6 hours, 6 minutes; for the 50 mg. chlorpromazine-treated 6 hours, 54 minutes; for the 5 mg. chlorpromazine and hypothermia group 6 hours, 26 minutes; and for the 50 mg. chlorpromazine and cooled group 7 hours, 36 minutes (fig. 4).

These results are affected by an uncontrollable variable in the experiment that two of the five forms of therapy produced. The amount of blood lost was essentially the same for the hypothermic, 5 mg. chlorpromazine-treated and control dogs, but it was greatly reduced in those receiving the autonomic blocking agent in large doses, either alone or when combined with surface cooling (table I). Also notable

TABLE I
EXPERIMENTAL HEMORRHAGIC SHOCK

	Initial blood pressure (mm. Hg)	Time to reach maximum bleeding volume	Maximum bleeding volume (cc./Kg.)
Control	93	1 hr.	51.8
Hypothermia	94	1 hr. 18 min.	51.4
Chlorpromazine 50 mg.....	80	3 hr. 24 min.	41.1
Chlorpromazine 5 mg.....	84	2 hr. 24 min.	52.1
Hypothermia + Chlorpromazine 50 mg.....	73	3 hr. 51 min.	42.4
Hypothermia + Chlorpromazine 5 mg.....	90	2 hr. 32 min.	53.5

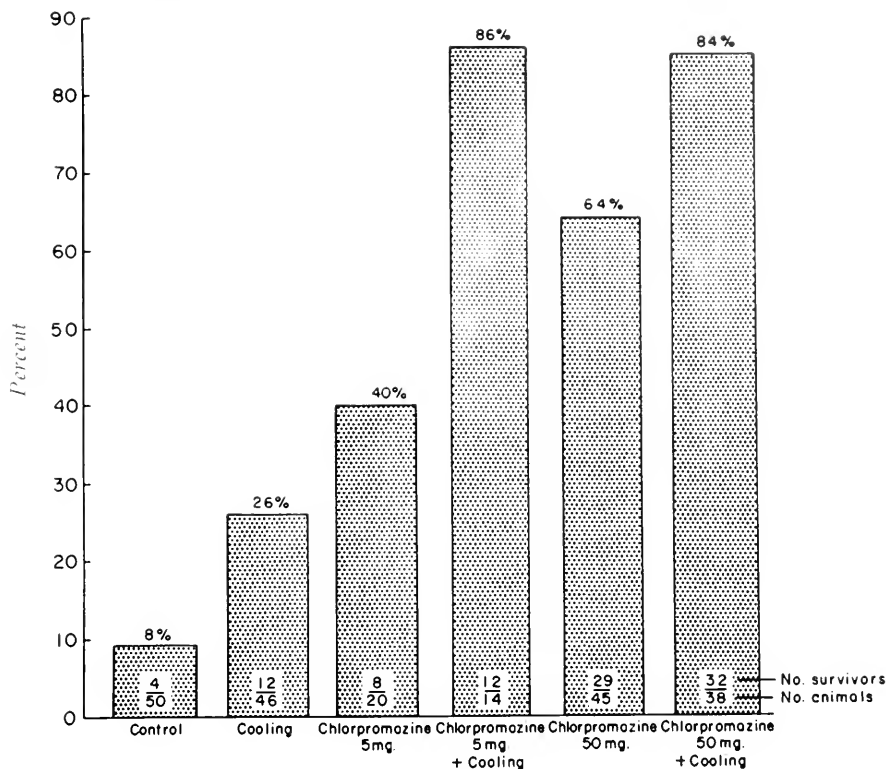


FIG. 3.—Experimental hemorrhagic shock, number of animals tolerating hypotension eight hours.

is the decreased rate of the blood loss in the dogs receiving the drug in large doses (table I). This decrease in amount and rapidity of bleeding in these animals may be a reflection of the initial hypotension. The average blood pressure at the beginning of the experiment was 93 mm. Hg in the control dogs; 94 mm. Hg in the hypothermic dogs; 84 mm. in the 5 mg. chlorpromazine-treated; 90 mm. in the 5 mg. of chlorpromazine and cooled group and 73 and 80 mm. Hg, respectively, in the group receiving the larger dosage of drug alone or in combination. The better survival rates in the 50 mg. chlorpromazine and cooled dogs as compared with those receiving the drug in large dosage alone is not entirely explained on this basis, for despite the lower initial blood pressure, both groups were bled almost identical amounts and in essentially the same period of time. This would suggest an actual augmentation of effect when surface cooling was combined with the drug. Other workers have pointed out the apparent better tolerance of the organism to hypotension produced by sympathectomy, anesthesia or autonomic blocking agents than to hypotension mediated entirely by acute hypovolemia, and perhaps the beneficial effects reflect a lesser volume of the hemorrhage.³⁴ This does not mean that the drug in large dosage had no beneficial effect on the shock experiment, but the results must be interpreted cautiously. Hypothermia and the smaller dosage of drug, however, augment compensation in another fashion, since the volume and rapidity of loss of blood in these animals closely approximated those of the controls.

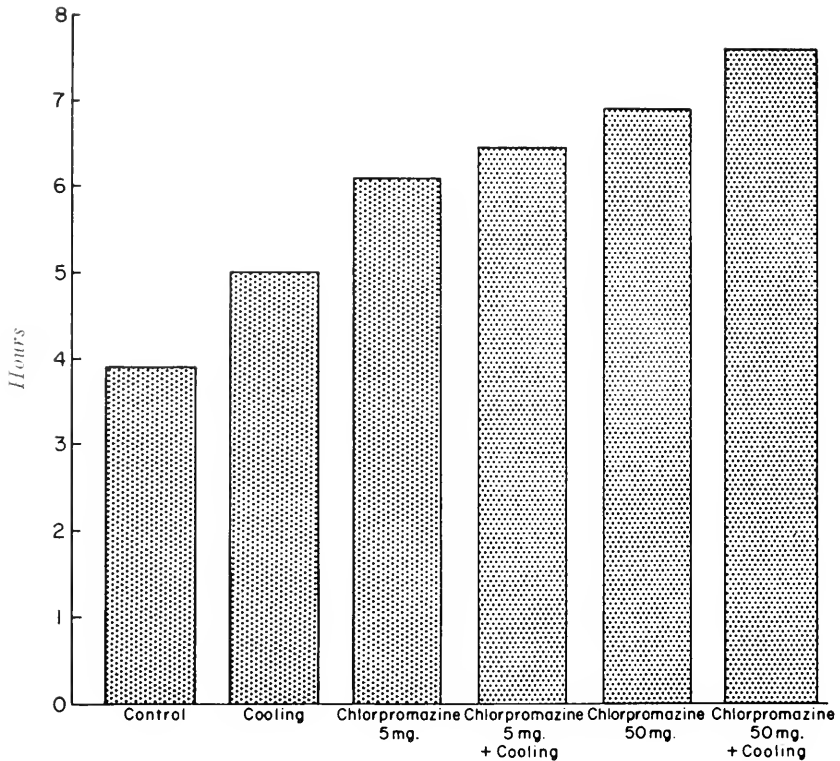


FIG. 4.—Experimental hemorrhagic shock, hypotensive time.

Discussion. The hemorrhagic shock preparation used in this experiment is fairly well standardized and has been uniformly associated with a high mortality in previous studies both in our laboratory and by other workers. Accordingly, it offers an excellent method of controlled evaluation of the various therapeutic factors employed in "artificial hibernation." Although there are numerous reports of the clinical application of hibernation to a wide variety of situations in which shock is believed to be affected beneficially, there is a paucity of data on its use under controlled experimental conditions. Jaulmes *et al.*⁴⁰ employing Wigger's method of inducing hemorrhagic shock, demonstrated an apparent protective action, but no attempt was made to ascertain the action of the individual factors, since both drugs and cooling were applied to the small number of dogs employed.

This study tends to support the concept that artificial hibernation does offer some protective action against "irreversibility" in experimental hemorrhagic shock, although the results should be interpreted cautiously. Whether applied separately or in combination, surface cooling and chlorpromazine produced a significantly beneficial effect upon the course of the shock, but the significance and mechanism of this alteration are not entirely clear.

Surface cooling preceding bleeding significantly increases survival and suggests an increased tolerance to shock as manifest by tolerance to the hypotension and blood loss. Approximately one-fourth of the animals were apparently compensated

at the end of the eight hours, and at termination had required only moderate replacement of blood for maintenance of compensation. These observations are perhaps the most significant of this study, since they most closely parallel the control animals in the amount and rapidity of the blood lost, and would indicate a variation in the animals' resistance to "irreversibility" rather than a variation in the experiment.

That cooling might be beneficial in shock was suggested much earlier than current interest would indicate, for it has been noted by several observers that animals subjected to burn, epinephrine drip, tourniquet release, and hemorrhagic shock demonstrated much greater resistance when the environmental temperature of the laboratory was low.^{1, 5, 12, 28, 42, 66, 69, 71} In his studies on the nervous system in shock, Remington⁵⁹ found that it was necessary to bleed the animals significantly more during the winter months. Allen,¹ in studies on tourniquet release shock, applied refrigeration to the constricted limb with an increase in survival of both animals and limbs, but he noted he had also inadvertently secured general body hypothermia. In comparing the effects of heat and cold in the prevention and treatment of shock, Blalock¹² found a significant increased tolerance to duration of shock when the animals were cooled but no increase in survival. Cooling was applied after shock was in progress, however, and the degree of hypothermia secured (average 25° C.) without supportive measures for respiration perhaps influenced the mortality adversely. These and other studies coupled with our results perhaps do not fully support the theory advanced that hypothermia has a protective action in hemorrhagic shock but do suggest the need for further inquiry into the problem.

The mechanism involved in the beneficial effects of hypothermia is not identified in this experiment, but may be due to one of several factors: first, it seems likely that the lowered metabolism and reduced requirements of the tissues for oxygen prevent irreparable damage to the vital centers responsible for maintenance of compensation. This is an appealing concept in that it carries prevention of peripheral stagnant anoxia further than one is able to do by correction of hypovolemia. Recent studies have demonstrated the ability of cooling to protect a variety of organs from the acute anoxia of temporary interruption of circulation.^{22, 54, 55} It has been suggested, since tissue repair continues during hypothermia, that the temporary maintenance of the animal allows capillary beds damaged by anoxia to improve enough to prevent irreversibility.¹² In this regard selective cooling of viscera might add information as to the organ most responsible for maintaining compensation.

The second explanation for the beneficial effects of hypothermia is also conjectural but is based on possible alteration of effective blood flow to various body compartments during shock. Selective vasoconstriction, vasodilation or both to organs vital to compensation may be accomplished by cooling.^{3, 32, 33, 63} Aside from the known local and reflex vasomotor responses to heat and cold,^{4, 56, 58} it has been demonstrated that the temperature of the blood entering and leaving different viscera varies.⁵⁶ Rodbard⁶² and D'Amato²¹ and others⁶⁵ have suggested that whole blood sequestered in various vascular channels would account for the unexplained reduction of plasma volume that occurs in hypothermia. The close correlation of the amount and rapidity of blood lost among the controls and among the hypothermia animals in our experiment would tend to discount involvement of any shunting mechanism.

The results obtained in animals receiving chlorpromazine were striking both in terms of survival and increased tolerance to shock. This agent, however, produced an alteration in the experiment which affects comparison of these animals with the control and cooled animals. As previously indicated, the autonomolytic action of the drug, when given in massive doses, reduced the vasoconstrictive response to such an extent that bleeding was diminished in rate and volume. Survival and tolerance figures then perhaps reflect only response to lesser stress, since it changes the method to one of tolerance to hypotension rather than to blood lost. When the dosage employed was small, however, a beneficial effect was secured with no alteration in the amount of blood extracted, although it was done somewhat more slowly.

The possibility exists that chlorpromazine possesses a pharmacologic property aside from its autonomolytic effect to account for its beneficial action, but this latter property seems to be the one of significance. Our results closely parallel those demonstrated by Remington^{57, 58, 59} and others using dibenamine, a drug with similar but more pronounced autonomolytic properties. When the drug was administered in doses that produced partial vasoplegia but still allowed some vasoconstrictive response to bleeding, Remington was able to reduce significantly the mortality of hemorrhagic and traumatic shock.^{58, 59} The noteworthy feature of these experiments is the ability to extract from the treated animals the same volume of blood as from the controls. The effects of the autonomolytic drug employed in this study, when used in small doses, seem to add support to his findings. It had been previously demonstrated that ether and spinal anesthesia, ergotamine and other autonomolytic drugs, as well as surgical sympathectomy, permit great tolerance to the hypotension that accompanies bleeding but reduce the tolerance to amount of blood lost.^{31, 48, 52} Fine²⁹ has apparently been unable to secure a protective action with dibenamine in the method employed in this study, but perhaps the dosage employed was excessive, as was the case in some of Wiggers' animals.⁷²

The combined use of hypothermia and chlorpromazine, simulating in the experimental animal the therapeutic regimen of "artificial hibernation" used clinically, produced striking results in regard to survival when compared with those secured from their separate application. This study suggests that the benefits are derived largely from the autonomolytic action of the drug employed since the bleeding volumes and tolerance to hypotension so closely parallel the drug-treated dogs, although the cumulative improvement due to the cooling cannot be excluded. When given in combination, administration of small doses of the drug seems as efficacious as larger dosage in improving tolerance to duration of hypotension, and nearly as effective in the improvement of survival.

Summary. In an attempt to determine the effect on shock of the various factors employed in "artificial hibernation," namely, hypothermia and the administration of chlorpromazine, these factors were applied separately and in combination to dogs subjected to "irreversible hemorrhagic shock" by the technique of Fine.

Two hundred and thirteen dogs were divided into five groups. Fifty animals served as controls; 46 were cooled to 31° C. before shock was induced; 45 and 20, respectively, were initially treated with 50 and 5 mg. of chlorpromazine and in 38 and 14 hypothermia was combined with 50 and 5 mg. of chlorpromazine, respectively.

Both immediate and ultimate survival were significantly improved by administra-

tion of all the listed factors, either separately or in the various combinations. The drug in large dosage combined with hypothermia proved the most effective. A protective mechanism was also suggested by the increased tolerance to hypotension in the treated animals. This improvement was noted both in terms of hypotension time and in the number of animals which were still compensated after 8 hours of shock and their experiment arbitrarily terminated. Combination of the drug, in either small or large dosage, with hypothermia proved to be most effective therapy.

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HYPOTHERMIA AND EXPERIMENTAL MYOCARDIAL INFARCTION*

CHARLES HUGGINS

We have been interested in the effect of pre-existing pathology on the safety of hypothermia, particularly within the range of temperatures employed clinically. As ventricular fibrillation appears to be the greatest hazard of general body cooling we thought it reasonable to investigate first the effect of hypothermia on dogs with heart disease. In particular, we chose to study dogs with recent myocardial infarctions.

Healthy male mongrel dogs were cooled to 28.5° C. by immersion in ice-water. They were allowed to equilibrate (usually between 24–25° C.) for one hour, and then rapidly rewarmed. This initial cooling served as a control on the health of the animal, and allowed us to obtain electrocardiograms for future comparison. One week after control cooling a thoracotomy was performed and the anterior descending branch of the left coronary artery was occluded, either by direct ligation and division, or by the insertion of a small polyethylene cannula into the artery at the same level. Large areas of infarction were consistently produced. Three to five days following the thoracotomy, the animal was again cooled exactly as before.

In the first experiment 24 dogs underwent control cooling. These animals were forcibly hyperventilated with pure oxygen throughout the procedure. Technical errors early in the experiment caused the death of two animals. Myocardial infarcts were produced in the remaining 22 animals and caused the death of 13. None of the nine animals surviving both procedures died from their experimental cooling.

We were surprised by these results and wondered if prevention of fibrillation was accomplished merely by forced hyperventilation with oxygen. Accordingly, a second experiment was performed which was identical with the first except that the animals were hypoventilated to the point of hypoxia. Seven of twenty dogs died from control cooling alone. Five of these animals fibrillated and two died with nervous system damage. Seven of the 13 animals which survived cooling were quadriparetic up to 48 hours. Myocardial infarcts were produced in the 13 dogs which survived control cooling and five lived to undergo later experimental cooling. Four of these five dogs cooled uneventfully although two exhibited transient quadriplegia afterward. Death in the fifth animal was probably related to a technical error, but must be evaluated as a fibrillation mortality.

All dogs which fibrillated show the same changes in the ST segment of the electrocardiogram that Dr. Hegnauer has demonstrated.

Our conclusion from this study is that in the dog the mere presence of a recent myocardial infarction, even though of large size, is not a major stimulus to ventricular fibrillation. Clinical application of this datum must be qualified by the realization that the human patient with a myocardial infarction has generalized coronary artery

* The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

disease whereas our dogs have otherwise normal hearts. This study suggests that if the potential benefits to be derived from an operation under hypothermia are great, and there is no evidence of current myocardial ischemia (by history or electrocardiogram), the history of a past uncomplicated myocardial infarction should not serve as an *absolute* contraindication to cooling.

TREATMENT OF THE SERIOUSLY ILL, FEBRILE PATIENT WITH SURFACE COOLING*

F. JOHN LEWIS, DEAN M. RING AND JOHN F. ALDEN

This paper will describe an effective and relatively simple method for lowering the body temperature of febrile, gravely ill patients. This idea is an ancient one, but the technique is new.

Though these patients have not been cooled to truly hypothermic temperature levels, inclusion of this discussion in a Symposium of Hypothermia can be justified in several ways. First of all, the metabolic changes that accompany cooling of a feverish patient to normal or slightly subnormal temperature levels mimic those attained when a normothermic individual is made moderately hypothermic. In both cases the pulse and respiratory rates decrease as the general metabolic processes slow down. These changes accompany a significant drop in temperature which has been at least 7 or 8° F. in the feverish patient. In the matter of equipment, too, cooling of the febrile patient is similar to the production of hypothermia in a normothermic patient. We have used the same cooling apparatus in both situations. Finally, introduction of this subject into a Symposium on Hypothermia provides an opportunity to bring up the topic of "Artificial Hibernation." This treatment, developed by Laborit and other French investigators,² suggests by its name that a profound hypothermia, like that of hibernating animals, is produced, while actually only a slight drop in temperature occurs.

Laborit and the many other European authors who describe their use of "Artificial Hibernation" give a number of drugs mixed to make a "lytic cocktail" and then add mild surface cooling. This system is used as an anesthetic technique, primarily, and good results have been reported, but there has been little enthusiasm for "Artificial Hibernation" in this country as yet. This may be due simply to difficulties in communication, but there may also be an attitude of scepticism toward the use of a mixture of drugs which, separately or in combination, do not actually produce a state resembling true hibernation. The chief effects of these drugs may be merely sedative and vasodilatory.

Shackman⁵ has analyzed the action of the "lytic cocktail" and he reports that these drugs do not produce a central effect on the temperature-controlling apparatus but only a peripheral vasodilatation similar to that produced by other vasodilators. This effect does not produce hypothermia in the unexposed patient. Another author from the British Isles, Dundee,¹ has in his animal experiments reached further interesting conclusions concerning the drugs of the "lytic cocktail." He found that deep anesthesia, curarization, or the "lytic cocktail" were equally effective in facilitating hypothermia and his data show, in addition, that chlorpromazine alone was as effective as the mixture of drugs in aiding surface cooling. Influenced by this work, we have used only chlorpromazine and one other sedative, phenobarbital sodium, while cooling our feverish patients.

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Our use of surface cooling for febrile patients arose directly out of our interest in hypothermia for intracardiac surgery.^{3, 4} The first patient we treated was a 29-year-old woman (U.I.I. No. 846504) who had just undergone an operation for an atrial septal defect complicated by mitral stenosis. She was considered to be a poor operative risk because of this additional lesion and also because she had mild congestive heart failure, but despite these complications she withstood the operation well. Her temperature rose to 104.2° F. a few hours after surgery, however, and with this her heart rate climbed to 140, her systolic blood pressure fell below 80, and she developed pulmonary edema. It was necessary to assist her breathing, with an anesthesia machine working through a tracheotomy tube, in order to avoid cyanosis. These measures did not seem to be enough to save her. We then lowered her body temperature rapidly to 96° F. by wrapping her in the same refrigerating blankets that had cooled her for the heart operation a few hours earlier. The effect was gratifying. Her pulse rate fell and her blood pressure rose slowly. After a few hours, it was possible to stop assisting her breathing. For several days it was necessary to keep her body temperature below normal. If the temperature was allowed to rise above 99° F. she would suffer an immediate and apparently detrimental increase in pulse and respiratory rates. After five days, cooling was no longer required and the remainder of her hospital course was untroubled.

In the one and one-half years since this first patient was successfully treated, we have used surface cooling to treat 24 other gravely ill, hyperthermic patients.

Method. The two essential features of the technique are surface cooling with a large refrigerating blanket and the administration of enough sedatives to prevent shivering. The patient is placed supine on a bed-sized cooling blanket (figs. 1 and 2) which consists of a long rubber tube sewn between two rubberized sheets. This tube runs back and forth the length of the blanket and a cold anti-freeze solution is pumped through it by a special machine[†] which can either cool or warm the solution, and consequently the blanket, to any desired temperature. At the start the blanket is cooled to 40–50° F. and occasionally in obese patients ice bags or ice chips are placed over the groins and axillae in addition. This intense surface cooling will cause shivering unless the patient is deeply comatose or properly sedated.

For sedation we have used chlorpromazine and phenobarbital sodium intravenously. When shivering first appears, 50 milligrams of chlorpromazine are given and if shivering is still present ten minutes later, 0.13 or 0.20 gram of phenobarbital sodium are given. In another ten minutes, if shivering still persists, an additional 50 milligrams of chlorpromazine are administered. This is usually enough, but further 50 milligram doses of chlorpromazine may be necessary if the patient is large or if he has been unusually active just before the cooling was started. In any case, enough is given to suppress all shivering—even the slight tremor that may be detected by palpating the pectoralis major muscles. In an occasional patient as much as 200 milligrams of chlorpromazine has been given during the first two or three hours of cooling.

After the temperature has started to fall less cooling and less sedation are required. It may be possible to stop administering sedatives altogether when the temperature has reached normal, but in some cases an occasional dose of chlorpro-

[†] Manufactured by Therm-O-Rite Products Corp., 17 Main St., Buffalo, N.Y.

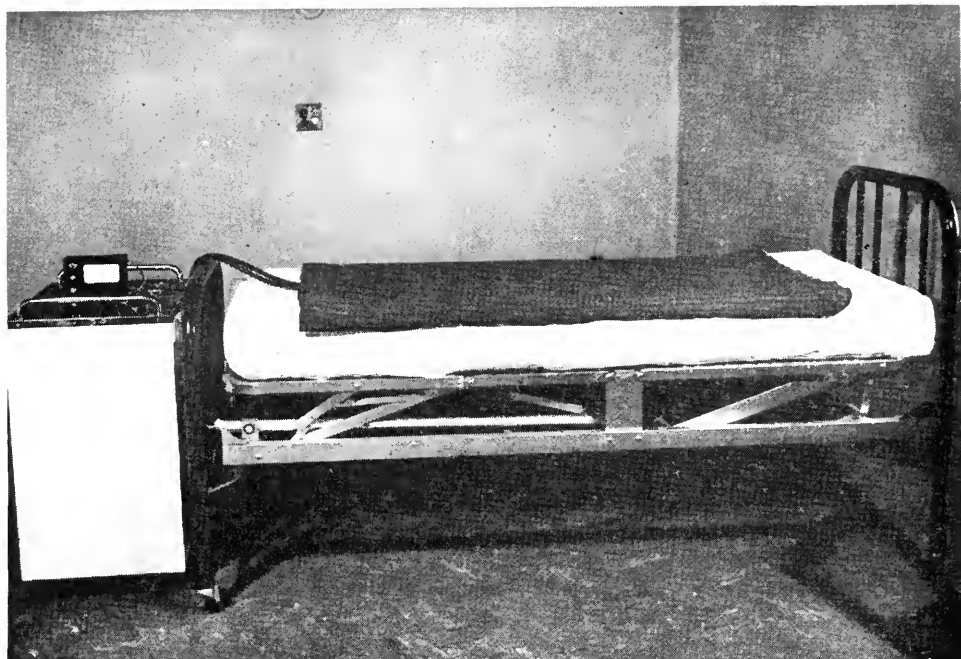


FIG. 1.—Photograph of the cooling apparatus. The refrigerating machine which cools the anti-freeze solution and pumps it through the blanket is shown at the left, and the large cooling blanket is spread out on the bed. The black box with the dial sitting on the refrigerating machine is the electrical thermometer.

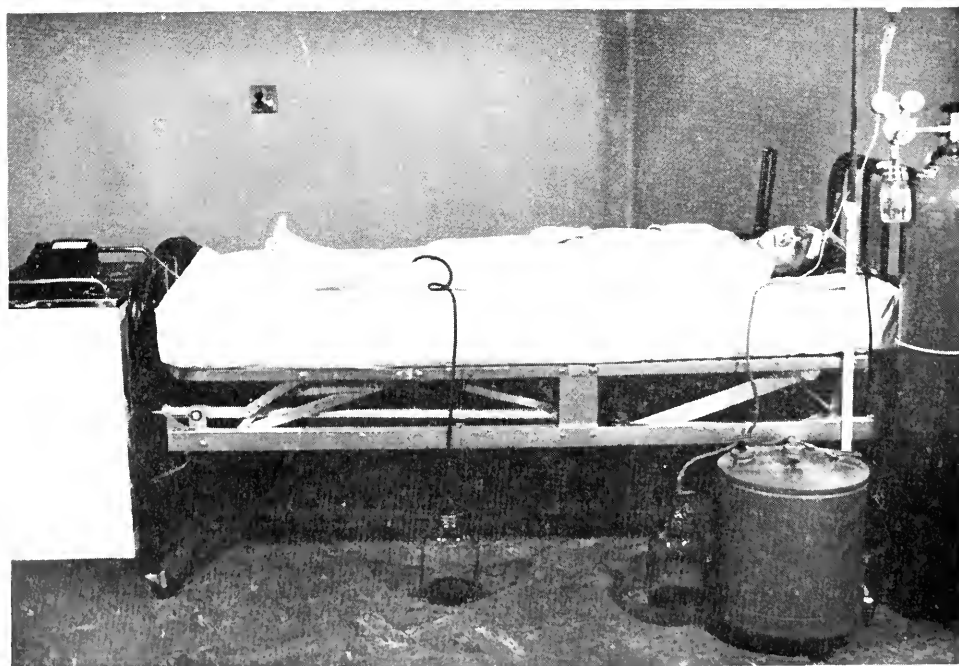


FIG. 2.—A single sheet has been placed over the cooling blanket and the patient is in position.

mazine is still required. As the body temperature falls below 100° F. the blanket temperature may be increased from its original level of 40–50° F. up to 60 or 70° F. and later, when the body temperature levels off at 95–98° F., the blanket may be warmed a little more. Our objective has been to keep the body temperature within a range of 95 to 98° F. To maintain this level, once it has been reached, a blanket temperature of 70 to 80° F. is usually adequate. It is surprising, at first, to find that the blanket at this temperature provides enough cooling in most cases after the first few hours. The blanket is at room temperature and it does not feel cold to the touch. It is effective, however, because the entire blanket and consequently a large area of the patient's skin is kept at this temperature due to the constantly circulating fluid in the coils of the blanket. The patient's back feels cool—as cool as the blanket—while in an ordinary bed the back would be hot.

It is not difficult to decide when cooling is no longer required. Any time the patient's temperature falls below the desired range the circulating pump is turned off and the patient is allowed to rewarm himself slowly. Then, if the body temperature begins to rise slightly above normal again, usually in the afternoon or early evening, the pump is turned back on. Cooling may be required intermittently in this fashion for several days, but finally, if the patient is to recover, it is no longer needed during any part of the day. When the rectal temperature drops below the desired level during cooling it is dangerous to rewarm the patient by heating the blanket above the skin temperature. By hurrying to rewarm the patient this way one is likely to bring the temperature up to a fever level again and the intense cooling and heavy sedation will have to be used once more, as at the beginning. It is much better to simply turn off the circulating pump when the temperature drops too low and wait patiently for the patient to rewarm himself.

Unwanted swings of temperature are avoided by watching the rectal temperature continuously so that appropriate changes in the blanket temperature may be made to counteract a slight rise or fall. For this close observation, we have used an electrical thermometer with a temperature sensitive thermistor kept at one position in the rectum.

Results. Twenty-five seriously ill patients with temperatures of 103° F. or higher have been treated with surface cooling. In most cases the method was employed only after more conventional treatments appeared to have failed. Prior to cooling the prognosis for recovery was poor in each case, and a number of the patients were failing rapidly. In fact, two patients whom we were asked to treat died while we were assembling the equipment. They are not included in the statistics. Of the 25 who were treated, 12 died and 13 survived. One of the 13 survivors died over one month after cooling had been stopped.

There was a wide range in age (6 to 84 years) but most of the patients were past middle age (median age for the group was 59). The sex distribution was almost equal (13 females and 12 males).

Each patient had a high body temperature but surface cooling brought it down to normal in every case. Rectal temperatures before treatment were 103° F. or over and the highest temperature was 107° F., in the oldest patient—an 84-year-old man who survived. It took a median time of three hours after cooling had been started to bring the body temperature down to 99° F. Thirty minutes was the shortest

time interval during which this initial temperature drop was achieved, and the longest was 10 hours. This was in a man who had not been adequately sedated at first. Simultaneously with the initial drop in temperature, the pulse and respiratory rates decreased, though they did not always drop to normal.

In some cases with low blood pressures cooling produced a significant rise. Eleven of the 25 patients had systolic pressures below 100 before cooling despite adequate blood replacement. In six of these 11 patients, the systolic level rose above 100 after the temperature had been reduced. Four of these six patients survived.

Severe abnormalities of consciousness or sensorium were common before cooling. Eleven patients were comatose and nine were semi-comatose or delirious. Among the 11 with coma, six recovered, one survived cooling but died more than a month after cooling had been stopped, and four died. Of those who were semi-comatose or severely disturbed, six died and three recovered.

In table I the mortality is shown for the various clinical groups into which the patients have been separated. Though these groups are small, it would appear that success has been greater among some types of patients than among others. There were more survivors among the head injuries, for example, than among patients suffering complications following major abdominal surgery for cancer. These cancer patients had complications, such as hemorrhage, peritonitis, and lung or intra-peritoneal abscesses. Reduction of the dangerous hyperthermia was not enough to solve their problems. Where fever was more clearly the major issue, as in the patients with brain damage, the results were much better.

Discussion. In one respect the method has been uniformly successful. It decisively reduced the temperature of febrile patients and it kept the temperature down effectively and without great difficulty. The technique is a more certain and controllable method for cooling feverish patients than the commonly employed nursing techniques of using alcohol sponge baths or applying ice bags. It is a better method of cooling because of two special features. The first is the refrigerating blanket. This blanket provides a convenient and accurately controlled way of cooling the entire bed surface and thus a large area of the patient's skin. The second important feature is the administration of enough sedation to prevent shivering while the temperature is being lowered. Chlorpromazine has been particularly effective in this role.

TABLE I
MORTALITY AMONG FEBRILE PATIENTS TREATED WITH SURFACE COOLING

Clinical type	Number of patients	Survived	Died
1. Brain damage: 3 head injuries and one cerebrovascular accident..	4	3	1
2. Major complications following general surgical operations (except abdominal cancer)	7	4	3
3. Major complications following operations for abdominal cancer..	5	1	4
4. Following thoracic operations.....	3	2	1
5. Following urological operations.....	2	2	0
6. Preoperative (too ill for surgery).....	3	0	3
7. Burns	1	1	0
Totals	25	13	12

So much for the clear-cut accomplishment of the technique—abolishing the fever. Much more important, of course, would be establishment of the treatment's merit in helping the patient to recover. But since this, like too many clinical projects, was not a controlled experiment, we cannot be certain and must for the present fall back on clinical impressions. We do have the impression, if not the conviction, that many of the patients were benefited and the treatment was not obviously detrimental to any of them. The temperature reduction seemed to help all of the survivors and for a few it may have had critical value. Moreover, the temperature drop appeared to benefit, temporarily, about half of the patients that died. When the drop in temperature was beneficial, the exhausting effect of a fast respiratory rate, a racing pulse, and purposeless muscular activity was overcome and the patient would then appear to rest quietly.

We plan to continue using this technique of surface cooling, but now in a controlled clinical experiment. Perhaps in this way we will be able to make a more objective analysis.

Summary. 1. The paper describes a technique for reducing the body temperature of seriously ill patients with high fevers. The patient lies on a large cooling blanket while chlorpromazine and phenobarbital sodium provide enough sedation to prevent shivering.

2. Thirteen of 25 patients treated, all with fevers over 103° F., survived.

3. The survivors all appeared to have been benefited and one-half of those who died may have been temporarily helped by the treatment.

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DISCUSSION

Dr. H. J. Kolff: Our experiences tend to confirm those of Dr. Lewis although our results are less favorable due to selection of more seriously ill patients.

In simple refrigeration, the patient is anesthetized and subsequently cooled. The defense reactions of the body set in, and oxygen consumption is increased, at least in the beginning.

Conversely, in artificial hibernation, the patient is given a combination of drugs intended to dampen certain parts of the central and the autonomic nervous systems.^{1, 2} The blocking is said to take place at the levels of cortex, midbrain, ganglia, and nerve endings.

The most important drugs used in artificial hibernation are derivatives of phenothiazine: Phenergan, Diparcol, and chlorpromazine. Phenergan, Diparcol, and

chlorpromazine have to a certain extent similar actions, although there are differences. Phenergan for example is an antihistaminic. Diparcol is supposed to be a bronchodilator and to inhibit bronchial secretion. Chlorpromazine is the most important of the phenothiazines in artificial hibernation.

I am unable to assess the relative virtues and activities of the many drugs used. It seems to be intuition that has guided the French. It is an approach that is widely criticized in this country; however, I think that we should reserve our judgment in this instance until we have had more experience and when we want to see whether "hibernation" has some virtue, why not follow their recommendations exactly?

Nine patients, all of whom were considered beyond recovery by more conservative methods, were treated with artificial hibernation.³ Uncontrollable deterioration, shock, spiking temperature, and restlessness were among the indications for hibernation. The technic of Laborit and Huguenard was followed as closely as possible. In seven patients a rapidly downhill clinical course seemed to be arrested, at least temporarily, and patients who seemed about to die lived 1 to 19 days during or after hibernation. Eight of the nine patients were more comfortable during hibernation. Such signs as spiking temperatures, ileus with distention, convulsions, extreme restlessness, and cyanosis gave way to controlled temperature, less distention, quiescence, and pink color. Two patients recovered temporarily but finally died from the underlying disease. It is concluded that artificial hibernation deserves further trial in patients with potentially curable disease who presently would succumb to overwhelming illness or during the struggle to overcome it.

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Dr. E. Calkins: Since many of these extremely febrile cases are also in circulatory difficulty, I wonder if you had any deaths which obviously followed intravenous injection of chlorpromazine, which reduces the blood pressure rapidly in some people.

Dr. Lewis: Some of the patients who died had a fall in blood pressure even after cooling. Of these eleven who had low blood pressure, some went on to die. Whether it was due to chlorpromazine or to a continuation of their lethal disease process, we really couldn't say. On the other hand, six of the eleven with serious vascular collapse recovered their blood pressure after the cooling. I am afraid I can't answer the question more definitely than that. We haven't felt that there was any clearly detrimental effect from the technique in any of the cases.

Dr. Henry Swan: I would like to ask what your thought is on the effect of mild hypothermia on the course of infection. Does the infective process seem to go faster or slower?

Dr. Lewis: This is a clinical study. A number of the patients had pneumonia. Some of them were old men with pneumonia. Some of them who recovered did

have serious infections. On a purely clinical basis, I can't conclude that reduction of the fever caused by infection aided progress of the infection.

Dr. Jean Henley: I would like to confirm this observation. We have watched two patients with temperatures up to 107° F. on several separate occasions; patients treated by chemotherapy into the carotid artery because of gliomas. Presumably the fever was due to brain damage. Each time, when all nursing methods had been tried to no avail, chlorpromazine was added intravenously. We could observe an almost immediate response by watching the dial of the thermister type thermometer we were using. We used small doses, repeating them every five to ten minutes until the temperature began to fall. The temperature would return toward normal and could be kept down by the usual nursing methods if chlorpromazine were given in doses of from 25 mg. to 50 mg. every four hours intramuscularly four or five times. When we found that this was efficacious, we tried Phenergan, a close relative of chlorpromazine and also a member of the phenothiazine series. Phenergan works perhaps a little less efficiently, but it has the advantage of not being toxic to the liver.

Lt. Col. Carl W. Hughes: We have studied the influence of hypothermia on infection at the Walter Reed Army Institute of Research. Adult white rats under sodium pentobarbital anesthesia were used. Experimental peritonitis was created in these animals by incising the cecum, expressing its contents and injecting hog gastric mucin into the peritoneal cavity.

Ninety-six per cent of the unoperated anesthetized animals survived cooling to 25° C. for 14 hours. The survival time of the uncooled rat with peritonitis was eight hours while survival time for the hypothermic rat with peritonitis was increased to almost 11 hours.¹

In order to evaluate the use of antibiotics in conjunction with cold in the treatment of peritonitis, rats were treated with 4 mg./kg. of dihydrostreptomycin administered intraperitoneally four hours after the induction of peritonitis. In these animals the survival time in the cooled and uncooled rats with peritonitis was increased to 16 hours.

Our data suggest that cooling may be of value in slowing the development of infection, but that additional therapy is needed for recovery.

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THE USE OF HYPOTHERMIA IN CARDIAC SURGERY*

HENRY SWAN

Because hypothermia slows the metabolic processes of the body, oxygen consumption and circulatory energy are reduced. This modality, therefore, suggested itself as being of potential value in the operative therapy of heart disease. Indeed, the possibility of this technique as a method for open intra-cardiac operation under direct vision during total circulatory occlusion was the stimulus which, for the past three years, has directed our experimental and clinical attention toward attempting to elucidate some of the alterations in physiology occurring in cooled and rewarmed individuals, and toward the elaboration of intracardiac operative techniques which could be safely performed by this method.

This report is concerned with an analysis of 24 patients who underwent standard closed cardiac operations, and 81 patients who underwent 84 open-heart operative procedures during hypothermia.

Preparation of patients for hypothermic anesthesia is essentially similar to any anesthetic procedure. Morphine, Demerol, barbiturates, and scopolamine are given for pre-medication. Induction is usually with ether. Two intravenous cannulae are placed to assure that this route for fluids or blood will be available. Electrocardiograph needle electrodes are connected and a rectal thermocouple inserted. Throughout the induction and cooling period a surgeon is available for immediate cardiac resuscitation if need arises. This precaution was instrumental in saving at least two patients who underwent circulatory arrest before thoracotomy had been performed.

When the patient is in second plane, third stage anesthesia he is placed in a tub of tepid water. The head and arms are held up out of the water. If shivering ensues, d-tubocurarine is given. When vital signs are stable, ice cubes are added to the water. Hyperventilation is deliberately performed throughout the anesthetic experience, except during circulatory occlusion.

The patient is removed from the tub when the rectal temperature has reached a point which is about two-thirds the desired fall. This figure varies somewhat, but the end temperature can be estimated in this fashion, usually, within a margin of error of one or one and a half degrees centigrade. To cool an infant requires about 10-15 minutes in the tub, while an obese adult may need as long as an hour or an hour and 15 minutes.

When the patient is removed from the tub, he is thoroughly dried. The pelvic area is wrapped with one-inch felt which is taped in place. A standard diathermy coil is then accurately placed, taking care that the patient is supported so his weight does not lie on the coils. The diathermy is used to counteract a tendency to over-drift in cooling, and to warm the patient immediately following completion of the cardiac procedure. Blood replacement is begun early and attempt is made to keep pace with the rate of loss. Indeed, transfusion slightly in excess of loss is considered desirable.

About one-half of the patients show auricular fibrillation when rectal temperatures

*This study was aided in part by a grant from the United States Public Health Service (11-1559 C), and in part by a grant from the American Heart Association.

are in the high twenties (C.). We do not consider this a serious development, and most will revert to sinus rhythm at about the same temperature when rewarming.

About five minutes before the moment of circulatory occlusion, further curare is given to prevent contraction of the diaphragm. A determination of blood pH is made at this time. We believe it desirable that the patient be in a state of respiratory alkalosis, with a pH of 7.5 or greater. During occlusion the lungs are allowed to collapse completely, and respiration is discontinued. The surgeon occludes the inflow of blood to the heart; then, after a few seconds, occludes the aorta about one inch distal to the valve and injects 0.8–1.5 cc. neostigmine 1:4000 into the base of the aorta so that it will perfuse the coronary system. After an additional 10 or 15 seconds, the operative manipulation is performed.

Upon release of circulatory occlusion, the lungs are again ventilated with oxygen, and hyperventilation resumed. The patient may receive only oxygen until the end of the procedure. If further anesthetic agent is needed it usually consists of 50–50 nitrous oxide-oxygen.

Diathermy is now begun, applied intermittently—one minute off, two on—to help prevent skin burns. Attempt is made to have the patient have an auscultable blood pressure of 90 systolic or above before the thoracotomy is finally closed, in order to avoid later bleeding when the hypotension of hypothermia rises to normal levels. Upon completion of closure, the patient may or may not be further warmed in the tub filled with water at 45° C., depending on temperature. The endotracheal tube is removed when spontaneous respirations appear adequate. The usual temperature of waking is about 34° C.

The immediate postoperative period is extremely critical. Evaluation of effective circulating blood volume and myocardial function is extraordinarily difficult. A few cases of severe shock occurred at this time. Blood volume studies are done at this time to compare with preoperative levels. Improved understanding of the state of the circulation immediately following cooling is badly needed in order to control this stage of hypothermia more intelligently.

In the management of hypothermia, we have emphasized the following safety measures on the basis of personal experimental and clinical experience. We believe that a sudden shift of blood pH from respiratory acidosis toward normal may incite onset of ventricular fibrillation and that a high $p\text{CO}_2$ sets the stage for the induction of cardiac arrest. For this reason, we deliberately strive for a respiratory alkalosis throughout the procedure.

We use neostigmine for the coronary perfusion on the basis of experimental data suggesting its value. Concomitant with its clinical use, the incidence of ventricular fibrillation fell markedly. In fact, we have had no patient undergo this complication in the last thirty cases.

The prevention of coronary air embolus is highly important. To this end, we make it a practice to occlude the ostia of the coronary arteries with a non-crushing clamp during the open portion of the procedure, and to evacuate air by flooding the heart with Ringer's solution before circulation is resumed. Clamping the coronary arteries removes all coronary blood flow throughout the occlusion period and, therefore, is probably undesirable from this point of view. The maneuver probably shortens the safe duration of occlusion. However, the risk of coronary embolism

is so great that, in our hands, it is one of the serious limiting factors relating to open-heart techniques. In order to fill the heart with the Ringer's solution, the incision in the heart must be positioned *at the uppermost portion of the heart*. This requires wide exposure and demands a bilateral thoracotomy with a sternum-splitting incision. It also limits the cardiotomy in our hands to a right-sided incision; we have not been able to devise a safe left-heart approach which places the incision uppermost. This technique has been effective in our hands to the extent that coronary air embolus has occurred only twice in this series. Both patients were resuscitated by pumping and massaging the air through to the venous side of the coronary circulation.

Cardiac resuscitation has been done in standard fashion using intermittent manual compression, electric shock, potassium chloride, calcium chloride, and adrenalin as appeared indicated. The diathermy is an important adjunct in warming the patient when attempting to revert ventricular fibrillation.

No preoperative drugs have been used to affect cardiac action, except that digitalis was given to patients in frank failure. Pre- and postoperative penicillin is routinely used.

INDICATIONS

In congenital or acquired heart disease for which standard closed operative techniques are planned, the indications for hypothermia have been the following. In cyanotic heart disease, it was thought that the reduction in oxygen demand would result in better oxygenation of the tissues. A blue child gradually becomes pinker as temperature falls. We consider operative risk to be improved under these conditions. In heart disease associated with severe tachycardia, the extremely rapid rate we consider *per se* as undesirable. A patient with so-called atypical patent ductus, with a large heart pounding at 170, changes to one whose heart is quietly beating at 90. We are not sure, because we have not had sufficient experience, whether heart failure may not also be an additional indication.

On the other hand, patients with valvular disease resulting in left ventricular hypertrophy and strain appear to tolerate hypothermia less well. Our experience with this group is very small as yet, and we have only very preliminary impressions. It may develop that for some cardiac patients hypothermia improves risk; for others it does not.

The main indication for hypothermia in this series, however, has been its use to allow total circulatory arrest in order to perform direct-vision intra-cardiac operations. Selection of patients was largely limited to congenital diseases for which pre-existing operative techniques had proven to yield poor or inconsistent results, for example, isolated pulmonary valvular stenosis, or those for which standardized methods had not yet been developed, for example, septal defects.

COMPLICATIONS

Postoperative evaluation of the state of the circulation for several hours post-hypothermia is extremely difficult. During this period, we lost four patients due to hemorrhage, which was unrecognized and therefore untreated. For this reason, warming with diathermy until blood pressure is obtainable before closing the chest

is now routine. In addition, if there is evidence of postoperative bleeding, re-exploration will, in the future, be more promptly done.

Postoperative thrombo-embolism was the cause of death in three patients, all adults with repair of large atrial septal defects. We feel that the very large pulmonary vascular tree associated with this disease may allow stagnation and intravascular clotting when the blood flow through the lungs is drastically reduced by repair of the lesion. For this reason, in such patients we are now giving postoperative anticoagulants in an effort to forestall this complication.

Cardiac arrhythmias, especially ventricular fibrillation, occurred with considerable frequency in the early part of our series. Even though these hearts were usually restored to a regular rhythm by resuscitative measures, the patients often died in the postoperative period. Reducing the parameters of circulatory occlusion has been one factor, we believe, in reducing the risk of this complication.

In figure 1 is seen the relationship of the degree of hypothermia to mortality rate. As can be seen, patients whose temperatures are lowered below 26°C . have a sharp rise in their risk.

In figure 2 is seen the relationship between the duration of total circulatory occlusion and mortality rate. It is clear that maintaining circulatory arrest beyond eight minutes also causes a marked rise in risk.

It might be argued that the patients who were cooled below 25°C . were those with the biggest, sickest hearts. We needed more time and, for this reason, sought deeper hypothermia. Be that as it may, the fact remains that we did not achieve a safe prolonged operative time in these patients by this means.

For these reasons, we have come to believe that the safe parameters of open-heart surgery under hypothermia as we now employ it are procedures which can be done through a right cardiotomy, in less than eight minutes of occlusion time, at temperatures above 26°C .

Except for one patient with cerebral embolus, no brain damage was experienced by any patient in this series. The degree of cooling appears adequate to protect the

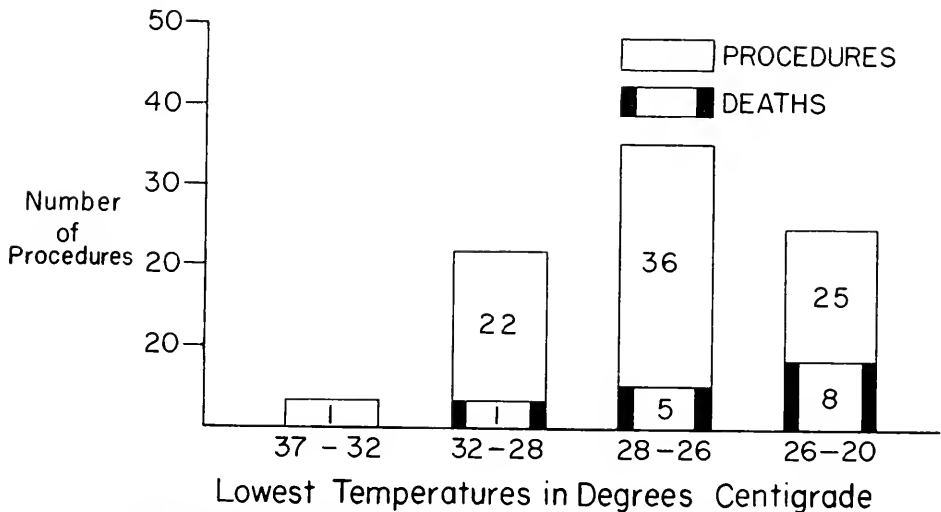


FIG. 1.—Mortality in relation to various temperature ranges (84 procedures).

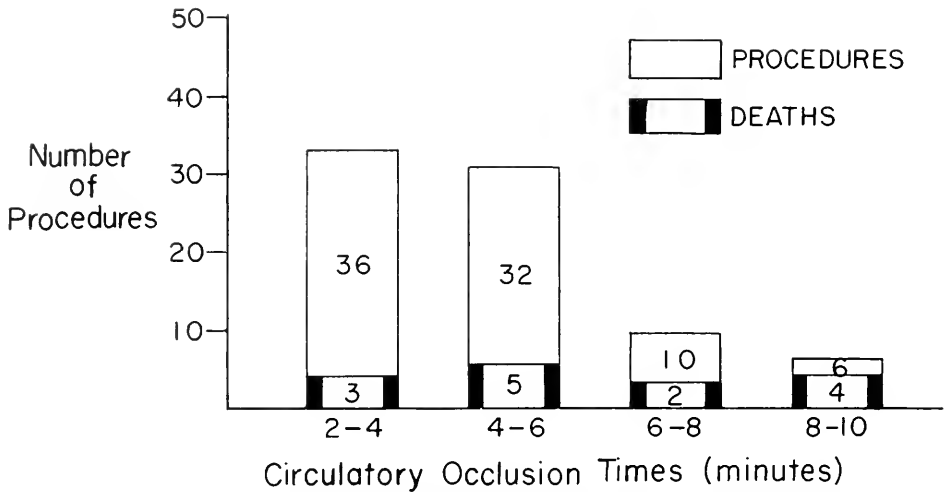


FIG. 2.—Mortality in relation to duration of circulatory occlusion (84 procedures).

higher centers for the periods of circulatory occlusion practised. A peripheral neuropathy, however, did frequently occur. This was carefully evaluated by our neurology service and the conclusion was reached that it was a complication of our method of surface cooling. The lesion was a direct injury to the peripheral nerves of the extremities due to cold. Time is an important factor. Few lesions appeared when the patient remained in ice water less than thirty minutes. For this reason, we now make it a practice to elevate the extremities above the water level after that period of time. Fortunately, all of the patients have experienced a return to normal function during the postoperative period.

CLINICAL EXPERIENCE AND RESULTS

In table I is seen the various diagnoses for which hypothermia was used in conjunction with standard closed operative procedures. In such small groups, the

TABLE I
CLOSED CARDIAC PROCEDURES

Diagnosis	Patients	Improved or cured	Unimproved	Died
Tricuspid atresia	3	0	1	2
Tetralogy of Fallot.....	2	2	0	0
Single ventricle	1	0	0	1
Idiopathic pulmonary hypertension.....	1	0	0	1
Patent ductus arteriosus.....	6	6	0	0
Aberrant pulmonary veins.....	2	1	0	1
Aorto-pulmonary window	1	1	0	0
Auricular septal defect.....	1	0	1	0
Aortic stenosis	1	0	1	0
Aortic regurgitation	2	0	0	2
Mitral stenosis and regurgitation.....	4	1	3	0
—	24	11	6	7

mortality figures are without significance. However, we were impressed with its apparent beneficial effect on the operative course of cyanotic children and in patients with patent ductus arteriosus and pulmonary hypertension.

In table II is seen our experience with 81 patients who had 84 open-heart operations with total circulatory occlusion. In this table, the postoperative results are based on objective data comparing pre- and postoperative studies. If postoperative studies are unavailable, the result is described as "too recent" even though the clinical course suggests a successful result.

This technique, we believe, is particularly adaptable for operations on the pulmonary valve and on the outflow tract of the right ventricle. A very deliberate careful plastic operation on the deformed valve can easily be performed in from 3 to 5 minutes of circulatory arrest. The danger of coronary air embolism is almost nil in view of the position of the patient, in table III, the operations on pulmonary

TABLE II
DIRECT VISION INTRA-CARDIAC PROCEDURES WITH CIRCULATORY ARREST
(81 patients, 84 procedures)

Disease	Patients	I. Pure (single) defects			Died
		Total cure	Partial cure	Too recent	
Pulmonary valvular stenosis.....	22	11	1	10	0
Pulmonary infundibular stenosis.....	2	1	1	0	0
Auricular septal defect (secundum).....	29	21	0	5	3
Auricular septal defect (primum).....	4	0	1	1	2
Ventricular septal defect.....	5	0	1	0	4
	62	33	4	16	9
II. Combined (multiple) defects					
Disease	Patients	Total cure	Partial cure	Too recent	Died
Tetralogy of Fallot:					
A. Valvular stenosis	6	0	6	0	0
B. Infundibular stenosis	7	0	5	0	2
Auricular septal defect:					
A. Pulmonary stenosis (trilogy).....	5	1	1	1	2
B. Ventricular septal defect.....	1	0	0	0	1
	19	1	12	1	5

TABLE III
OPERATIONS FOR PULMONARY STENOSIS

Disease	Patients	Total cure	Partial cure	Too recent	Died
Isolated valvular stenosis.....	22	11	1	10	0
Isolated infundibular stenosis.....	2	1	1	0	0
Valvular stenosis with tetralogy.....	6	0	6	0	0
Infundibular stenosis with tetralogy.....	7	0	5	0	2
Trilogy of Fallot.....	4	1	1	1	1
	41	13	14	11	3

valve and infundibulum are recapitulated. As is apparent, the only death following valvular operation occurred in a patient in whom there was an associated large atrial septal defect, the so-called Trilogly of Fallot. This infant died of some form of circulatory failure 16 hours following operation. This anomaly poses special problems and we are still uncertain whether one should repair the septal defect or the valve first, or should one attempt both at the same sitting. For resection of infundibular stenosis, one must be careful not to resect too much. In one patient, with Tetralogy, for example, following infundibular resection, a large aneurysmal dilatation of the thin-walled *infundibular chamber* occurred. Subsequent attempt to repair this complication was fatal. The other death occurred as a result of staphylococcic septicemia following a two week period of anuria. This patient underwent ventricular fibrillation during surgery, and a period of hypotension presumably occurred during resuscitation. This is the only death in this entire group in which hypothermia itself was considered to be contributory.

Atrial septal defect (table IV) is also well-managed by direct vision suture, using cooling to allow circulatory arrest. Particularly satisfactory are those patients with the so-called secundum-type lesion. A continuous suture is extremely effective in obtaining complete closure of these lesions, while aberrant pulmonary veins can be easily positioned to the left of the closure, except those which enter into the superior vena cava itself. Primum-type lesions or atrio-ventricularis communis are more difficult technically to manage within the allotted time limit. One of the deaths resulted from hemorrhage immediately postoperatively following attempt to repair atrial septal defect after conclusion of pulmonary valvuloplasty in a patient with Trilogly. As mentioned previously, three deaths were associated with thromboembolism, presumably of the pulmonary vascular tree, either arterial or venous. We believe for adults, therefore, that anti-coagulants should be given for a period of two weeks following repair of atrial septal defect. The other death occurred on the artificial kidney 12 days postoperatively, the anuria presumably due to transfusion reaction.

Ventricular septal defect, on the other hand, is a lesion of sufficient complexity anatomically to render the technical repair too difficult to accomplish safely within the current eight minute time limitation. As can be seen, only one of five patients survived such attempts. Two others had successful closure, but died a circulatory death within a few hours postoperatively. The other two were technical failures. This experience led us to abandon this procedure until longer periods for open operation became safely available, either by use of extra-corporeal circulations, or by better application of hypothermia.

TABLE IV
OPERATIONS FOR ATRIAL SEPTAL DEFECT

Disease	Patients	Total cure	Partial cure	Too recent	Died
Auricular septal defect (secundum).....	30	21	0	5	4
Auricular septal defect (primum).....	4	0	1	1	2
Trilogly	2	1	0	0	1
	—	—	—	—	—
	36	22	1	6	7

Summary. 1. Experience with 105 patients undergoing cardiac surgery during hypothermia has been described and discussed. Of these, 81 had direct-vision open-heart operations during circulatory arrest.

2. As currently applied in our hands, the safe parameters for open-heart operations appear to be a right heart cardiotomy, hypothermia not deeper than 26° C., and circulatory occlusion not to exceed eight minutes.

3. The chief causes of death have been ventricular fibrillation, post-operative hemorrhage, and delayed thrombo-embolic phenomenon. The methods currently being adopted to overcome these difficulties are discussed.

4. For cyanotic patients and those with severe tachycardia, cooling appears to improve operative risk when standard closed operations are performed.

5. Pulmonary valvular and infundibular stenosis, and atrial septal defect, especially of the secundum variety, are effectively treated at low risk by direct-vision repair. At the present time, we consider this method the treatment of choice for these lesions.

DISCUSSION

Dr. F. J. Lewis: Those of us who use hypothermia in Minnesota have used a slightly different technique. Although we strive to avoid respiratory alkalosis and Dr. Swan to produce it, there is some similarity, of course, because we both attempt to maintain a constant pH level. That may be more important than anything else in the technique. Except for that difference, most of our experience has agreed with Dr. Swan's, and I think that hypothermia provides, mechanically, the simplest method at the present time for doing open heart surgery. It provides the driest operative field for this type of operation, and it provides the best way at the present time, certainly, for doing open operations on adults where with heart-lung machines and other techniques the problem is quite a bit more complicated than it is in infants. In our own series of cases we have operated with success on adult patients as old as 61.

As to what operations you can do with hypothermia, I think that problem is obviously still unsettled. With a careful exploration of the heart with the finger before the open cardiotomy, and a carefully rehearsed technique, a great deal can be accomplished in seven minutes or less. For example, we recently operated on a patient with total anomalous pulmonary venous drainage; all the pulmonary veins ended in the right atrium. To repair this we made a large atrial septal defect and reconstructed the right atrium. The operation took eight minutes. Apparently the results have been completely successful. Further examples can be given. Recently we also operated on a tri-atrial heart under direct vision during hypothermia, and that operation took but three and one-half minutes. Our average time for the atrial septal defects has been four and one-half minutes. The high defects have taken us the longest to repair.

Dr. Jerome H. Kay: In order to prolong the time during which we can work inside the right ventricle, Doctors Robert Gaertner, James Isaacs, Richard Dever, and I have perfused the head and heart in a group of 157 animals.

In all of these animals the right ventricle was open for 15 to 30 minutes. We collected arterial blood from the femoral artery of donors and added 40 milligrams

of heparin to each liter of blood. The blood was perfused from an ordinary 2 liter graduated cylinder with the use of a sigma pump.

The technique employed consists of cooling these animals to a rectal temperature of 32 to 34° C. The right brachial (subclavian) artery is temporarily occluded distal to the internal mammary artery. A catheter is inserted into the internal mammary artery in order to take pressures during the period of bypass. A systolic pressure of 80 to 120 millimeters of mercury is maintained during the period of bypass.

The left brachial (subclavian) artery is occluded temporarily. The superior cava is occluded around a catheter that has been inserted through the azygos vein. Blood is drained from the superior vena cava by gravity. The inferior vena cava is occluded and the aorta cross-clamped distal to the origin of the left brachial artery. The sigma pump is turned on and blood is perfused in a proximal direction into the right common carotid artery. The blood, therefore, is pumped into the arch of the aorta. It can only perfuse the heart through the coronary arteries and the brain and head through the left common carotid artery.

We have used the right subclavian artery for the site of perfusion instead of the carotid artery in some dogs, and the results have been the same.

The last 20 dogs were cooled to a rectal temperature of 32 to 34° C. and the right ventricle was open for 15 to 30 minutes. The aorta was cross-clamped for 30 to 37 minutes.

In these dogs none of the 20 hearts fibrillated. Seventeen of twenty dogs are long-term survivors. The other three dogs died within the first 48 hours postoperatively. Gross and microscopic studies revealed pulmonary congestion.

The method described is safe and allows open heart surgery in the dog for periods of time up to 30 minutes.

During the past year Dr. Robert Gaertner and I have used hypothermia in more than 250 dogs in order to perform intracardiac procedures. All of these animals had inflow occlusion. Early in our experiments we cooled the dogs to a final rectal temperature of 20 to 25° C. and maintained inflow occlusion for 10 to 12 minutes. It soon became evident, however, that with inflow occlusion for longer than 7 or 8 minutes, the mortality rate was extremely high. We also noted that the incidence of ventricular fibrillation was very high at temperatures lower than 30° C. Temperatures of 30 to 32° C. safely protected the brain against damage for periods of inflow occlusion of 8 minutes. The incidence of fibrillation with inflow occlusion for 8 minutes at 30 to 32° C. was low and the recovery rate high. We therefore recommend that procedures requiring inflow occlusion be performed at 30 to 32° C. and that these procedures require less than 8 minutes.

Dr. I. K. R. McMillan: Two or three years ago we noticed something which at the time was regarded as ridiculous, namely, that we lost a lot more dogs from ventricular fibrillation in the summer than in the winter. This was in England, but Dr. Swan has had the same experience, and it was also reported by Cookson about five years ago. It has occurred sufficiently often in our experience that I think it is a matter that needs investigating. It is one of the interesting side issues of hypothermia which is little discussed, and perhaps my comment may stimulate others to add their observations.

Dr. William P. Longmire, Jr.: We have recently reviewed 100 cases in which

various degrees of hypothermia were utilized, and have classified these cases in three different groups.

The first group was called "The Controlled Temperature Group." In cases from this group, the temperature ranged from 34° C. to 30° C. The second group, in which temperatures ranged from 30° to 25° C., was called "The Moderate Hypothermia Group." The third group, which was labelled the "Deep Hypothermia Group," included those cases in which temperatures went below 25° C.

Most of these cases did not involve an interruption of the blood flow through the heart. They were, for the most part, closed cardiac procedures, and hypothermia was utilized because of the severe nature or character of the disease process.

We attempted to analyze these cases with regard to the ill effects of hypothermia alone. This was exceedingly difficult to do, particularly with respect to the cases in the deep hypothermia group, since many of these patients had conditions which were essentially incompatible with life.

Of the 10 cases in the deep hypothermia group, only three cases survived the immediate postoperative period, and there was only one long-term survivor.

There were two patients who exhibited severe postoperative bleeding which might possibly have been correlated with the degree of hypothermia. One of these patients was re-explored and found to have multiple areas of bleeding for which little could be accomplished. The other patient was treated merely by repeated aspirations, and subsequently survived the procedure. Similar problems have been encountered in cases of this type without the use of hypothermia.

Our only conclusion was that in this series of 100 cases there was no death clearly attributable to the use of hypothermia alone; nor were there any complications that might not have occurred had the hypothermia not been used.

Dr. Jean Cahn: I think that in operations on the bloodless heart there is a problem if the duration of the arrest of the circulation is over 20 minutes. We have two possibilities: (1) to infuse oxygenated blood into the occluded aorta so as to prolong the circulation into the coronary system; or (2) to be not inhibited by this problem and to consider that it is possible to operate on the bloodless heart for a period of 25 minutes without any blood supply into the coronary system. In fact, that is possible; but yesterday, when I told you that it was possible to arrest the blood circulation for 25 minutes without any ventricular fibrillation because of sino-atrial blockade, I gave you only 50 per cent of the problem and of the solution.

To resuscitate the heart after 25 minutes of arrest of the circulation, we must inject into the right chamber before the release of the caval clamping a mixture of A.T.P. and cytochrome-C. Under those conditions it is possible to re-establish the normal beat of the heart after only 30 seconds to one minute of cardiac massage. The cardiac massage must be done carefully. We have only to push out of the right cavity the blood and the mixture of A.T.P. and cytochrome-C.

Lt. Col. Carl W. Hughes, MC, U.S.A.: In keeping with the constant concern with ventricular fibrillation in the hypothermic animal, it was interesting to note in the film by Drs. Andjus, Smith, and Lovelock that in the supercooled animal, consideration was given to rewarming the heart faster than the rest of the body. At the Walter Reed Army Institute of Research, in a study of the tolerance of the hypothermic normal dog's heart to ventricular fibrillation, adult dogs were cooled until spontaneous ventricular fibrillation occurred. The animals were allowed to

fibrillate for various periods of time. These dogs were found to survive 60 minutes of ventricular fibrillation with complete recovery. After 75 and 90 minutes of fibrillation, survivors were found to have only a mild, persistent hindquarter weakness. Resuscitation in these animals was not a problem, but as the periods of ventricular fibrillation were lengthened to three, four, and five hours, resuscitation became increasingly difficult.

Resuscitation was accomplished by removing the animal from the ice water bath, placing the animal on a warm water mattress, opening the chest and irrigating of the pleural spaces with sterile saline at 45° C. With each 1° C. rise in rectal temperature a single defibrillation shock of 200 volts was administered to the heart for a period of 0.13 second. This routine was continued until defibrillation occurred and the animal had established its own adequate circulation. This warm saline irrigation, which we considered an extremely important factor in converting the fibrillating heart, was continued about the heart until a rectal temperature of 28° C. was reached.

Dr. Swan: I agree with Dr. Longmire that it is difficult to evaluate clinically what happens when one is dealing with a very sick patient and the patient dies. To try to implicate any part of the procedure is almost impossible. I hope that many others as well will think about and attempt to evaluate this problem.

I would like to thank Dr. McMillan for his comment, because in our laboratory, at least, we have found that the time of the year has a profound effect upon our experimental results. We in Denver have hot summers and fairly cold winters. We do not have the advantages of having an air-conditioned, temperature-controlled animal room, and our dogs are subjected in the wintertime to cold nights.

I believe this concept of pre-conditioning is of considerable importance in experimental hypothermia, and that it is one of the major causes why there is a great variety of results obtained in different parts of the country in studying this problem.

REVIEW AND APPRAISAL OF PART IV

R. D. DRIPPS

Induced hypothermia has been applied in the following fields:

Protection against ischemia. Reduction of body temperature can protect against diminished or absent blood flow. For this reason hypothermia has been used during interruption of the blood supply to the whole body (e.g., during open-heart operations), or to parts of the body (e.g., brain, liver, kidney, and gastro-intestinal tract). The degree of protection has not been defined for various temperatures nor for all tissues. These parameters must be outlined.

Whether low-flow or high-flow pump-oxygenators will replace hypothermia as the method of choice for cardiotomy remains to be seen. The tendency in certain clinics is in this direction. Interestingly enough, hypothermia is not recommended as an adjunct to pump-oxygenators by most workers.

Specific use in neurosurgery. The reduction in brain size during hypothermia should have a great appeal to neurosurgeons. If the method can be made safer it may have widespread applicability for craniotomy for this reason alone.

Use in "shock." As expected, a certain degree of protection against hemorrhagic shock has been demonstrated in animals when hypothermia has been used prior to bleeding. In operations known *in advance* to be associated with a large blood loss and a high incidence of shock this observation may find application. The value of hypothermia in man once shock has become established has not been documented. If shivering could be prevented there would appear to be theoretical grounds for permitting the body temperature of the shocked individual to fall, but studies on this have not been reported.

Effect on the course of infection. The few data which are available suggest little significant alteration of the course of infection. The influence of hypothermia on the formation of antibodies and other responses to infection deserves inquiry. The value of reducing the body temperature of markedly febrile patients has been recognized for centuries. Therapeutic application of this should be more widespread in clinical practice. The use of cooling for three to 10 days has been explored by some European workers in man and by a few investigators in this country in animals.

A number of clinical problems remain unanswered. Can the clinical impression that a surgical patient after hypothermia has a smoother postoperative course and appears less stressed than one operated upon at normal body temperature be verified by objective means? Observations of this sort tempt one to explore induced hypothermia rather than conventional methods of anesthesia for operations on desperately ill patients, regardless of the procedure contemplated.

What is the incidence of bleeding during and immediately after hypothermia? Is the reduction of platelets described in this symposium responsible for this, or are other abnormalities of the coagulation process present? Is there a greater tendency towards fibrinolysis?

The effects of changes in blood viscosity have received little attention.

The combination of induced hypothermia and the deliberate production of hypotension has been suggested. Data indicate the possibility that Arfonad may minimize the reduction of platelets (Helmsworth) and may protect against ventricular fibrillation (Riberi *et al.*). Use of this drug deserves further evaluation, as does the concept that hypothermia may reduce the hazards of a lowered arterial pressure. Might continuous spinal anesthesia be worthy of a trial during hypothermia?

PART V

PROBLEMS IN METHODS OF INDUCING HYPOTHERMIA BY EXTERNAL COOLING

WILLIAM H. MULLER, JR. AND J. FRANCIS DAMMANN

Ideally, a method for lowering body temperature should be simple, inexpensive, readily available, should permit one to cool at various rates with accurate control and should allow one to rewarm the subject without having to make extensive alterations in the apparatus. The methods in current use for inducing hypothermia by external cooling will be reviewed from these standpoints.

Immersion in cold water. This technique has been one of the most popular to date. It consists of bringing a tub partially filled with water and crushed ice into the operating room and immersing the patient in it. The temperature of the media is in the neighborhood of 4° C. and cooling is accomplished rapidly. Swan,^{1,2} Bigelow,³ Hegnauer,^{4, 5} and others have used it in preference to other methods. Its advantages are that it is simple, rapid, inexpensive, and readily available. The cold medium is evenly distributed over the body surface and makes cooling more uniform. Its disadvantages are that it is somewhat cumbersome, the patient must be moved from the tub to the operating table which, in the anesthetized state, can be accomplished but presents greater difficulties than when the patient is cooled on the table. Cooling must be virtually complete at the time the patient is positioned on the operating room table unless other adjunct means are available to drop the temperature to lower levels if desired. Another disadvantage is that if cardiac arrest should occur during the cooling period the patient would probably have to be moved from the tub to the operating table before cardiac resuscitation measures could be begun.

Application of ice bags. This method has been used extensively by Bigelow,⁶ Shumacker,⁷ Kaplan,⁸ and Scott⁹ (fig. 1). It is simple, inexpensive, and readily available. It allows one to begin the operation before the desired temperature level is reached. This method also distributes the cooling medium over a relatively large surface which is desirable for rapid cooling. The disadvantages are that accurate control of the temperature is lacking. This is especially true if one begins the operation before the maximum temperature drop is reached and leaves the patient on a bag of ice during the beginning of the operation. When the bag is removed, the underlying sheets and mattress are often wet and cold and the temperature may continue to drop for a period of time in excess of the expected drift. The plastic bags often develop leaks and as the ice melts water accumulates and makes the operating area messy. One of the more serious disadvantages is that fat necrosis has developed in infants cooled by this method. Collins, Spellman, and Scott,⁹ who first reported this complication, noted that the chemical composition of fat in the



FIG. 1.—Application of ice bags to infant undergoing cardiac surgery.

infant is different from that of the adult. According to Langer,¹⁰ the comparative concentration of fatty acids in infants and adults is as follows:

Newborn.	Oleic 67.75%	Palmitic 28.97%	Stearic 3.28%
Adult.	Oleic 89.80%	Palmitic 8.16%	Stearic 2.04%

Oleic acid has a much lower melting point than the other constituents and the small change in the relative proportions of these fatty acids results in a considerable change in the melting point of the neutral fat. It was postulated, therefore, that because of this, even slight changes in the temperature of the infant's fat can cause solidification and subsequent necrosis.

Blankets with coils containing a fluid. In this technique the temperature of the fluid circulating through coils can be readily regulated (fig. 2). One can thus cool or rewarm during an operation. The equipment is expensive and somewhat cumbersome; the rate of cooling is relatively slow. During rewarming skin burns may occur if the warming fluid is too hot.

Air cooling by cold air chamber. A cold air chamber has been used by Cookson, Bailey, and associates.¹¹ It has not received widespread acceptance in this country. The method is cumbersome; the rate of cooling is slow. Frostbite or pressure gangrene is likely to occur in the digits and unprotected parts of the body. If these are wrapped the rate of cooling is even more slow. Adams-Ray¹² has recently reported a method of air cooling which is more rapid and which apparently avoids these hazards (see Adams-Ray, Discussion, pp. 430 ff.).

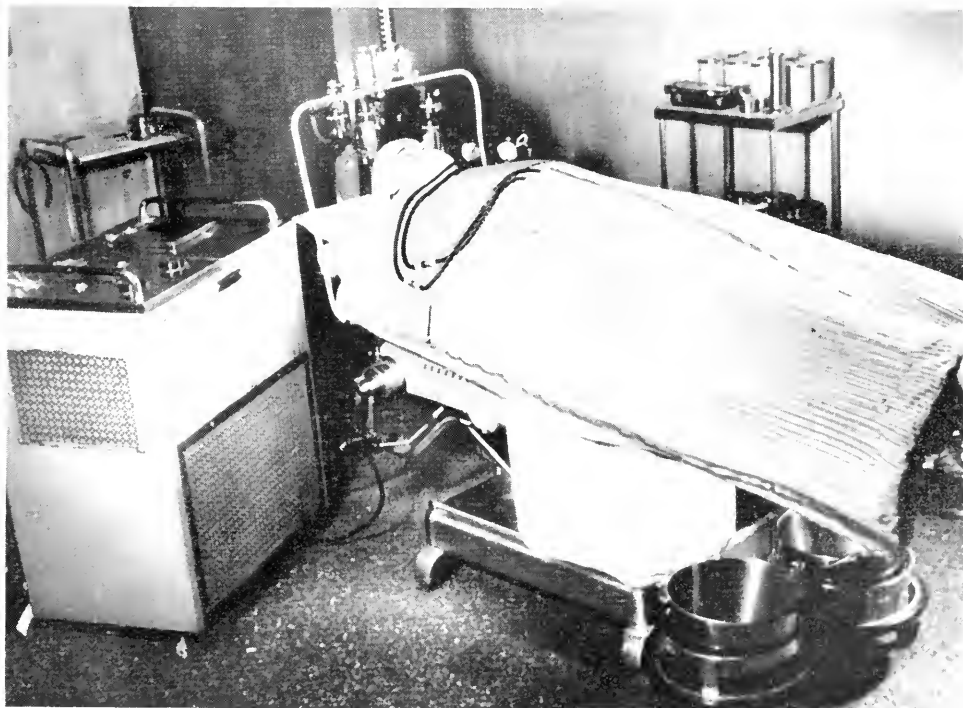


FIG. 2.—Production of hypothermia by circulating blanket.

Pleural perfusion. Pierpont and Blades¹³ have induced hypothermia by perfusing cold saline into the pleural cavity. During a thoracic operation such a technique could be readily applied. It may also be quite helpful in rewarming (see Hughes, Discussion, p. 412). It has a disadvantage in that, generally, it is not as rapid as other methods¹⁴ although Jaeger,¹⁵ using a closed circulating system, found that the temperature could be decreased about 1° C. every five minutes. It does not seem to be this rapid when cold saline is introduced into the open chest through a thoracotomy wound during an operation.

Peritoneal cooling. Jaeger¹⁵ has used peritoneal cooling in animals. One mushroom catheter was introduced into the left flank to serve as the point of entry for the coolant fluid while a sump drain was introduced into the right lower quadrant for removal of the fluid. The intrapericardial temperature was 1.1° to 4.4° C. lower than the rectal temperature. This was probably due to cooling of the blood flowing not only through the vena cava but through the portal vascular bed and other abdominal viscera returning blood to the heart. He thought the advantages of such a method were that cooling and rewarming did not interfere with the opening and closing of the chest or performance of the operative procedure. It seemed to reduce the temperature as rapidly as other methods. One animal died 48 hours after the operation from an intestinal hemorrhage and another dog, sacrificed at that time, showed, in the small intestine, punctate sub-serosal hemorrhage and edema of the mucosa. In addition, one wonders about the introduction of infection and the development of intestinal adhesions subsequent to this method of hypothermia.

Intragastric balloon. This method has been investigated by Khalil and MacKeith.¹⁶ They introduced a balloon through the esophagus into the stomach. The balloon was filled via a connecting tube with 250 ml. cooled water (4° C.). Body temperature in rabbits could be lowered to 25° C. in two hours. The method might conceivably be useful in infants and small children. It might have an advantage over skin cooling in that it would not be affected by cutaneous vasoconstriction occurring in response to cold. In animals profuse salivation and extrasystoles were noted. There are no data as to whether the heart might cool more than the rest of the body. This would be undesirable.

Rewarming. Rewarming may be accomplished by a number of methods. Immersion in water at a temperature of 40–42° C. is useful. It is rapid, simple, and controllable. It has the same disadvantages as immersion cooling. As already indicated the blankets containing coils can also provide warmth.

Diathermy has been used by Bigelow¹⁷ and Swan.² The chief advantage of this method is that it appears to minimize tissue gradients between the superficial and deep tissues because of its deep heating characteristics. In experimental studies, Bigelow and associates¹⁷ did not encounter vascular collapse. This might be attributable to a more uniform heating of deep and superficial tissues. Burns occur from this method, however, if there is inadequate insulation between the coil and the patient. One-half inch of rubber was alleged to provide sufficient insulation to permit satisfactory heating and prevent burns.

Air warming has been used by a number of investigators. It is accomplished by placing the patient in bed under a heat cradle. It is most useful when the tempera-

ture has been reduced only moderately because it is slower than most of the other methods.

Mechanics of hypothermia. In order to answer certain questions regarding the mechanics of hypothermia, fifteen groups using hypothermia were polled.

1. *Rate of cooling.* All but one of the 15 favored rapid over slow cooling. Most gave as the reason convenience for the surgeon and the operating teams. A more concrete advantage of rapid cooling is that the period of anesthesia is reduced. It was also felt that the incidence of frostbite and possible fat necrosis is reduced by rapid cooling. Another important reason for cooling rapidly involves alterations in coronary blood flow. Berne²⁰ has found in experimental animals that from 39.5° to 28° C. the aortic pressure decreased slowly, whereas below 28° C. it dropped sharply. In contrast to this, the coronary blood flow decreased precipitously in the early stages of hypothermia, declined more gradually as the blood pressure fell between 33.5° and 21.5° C., and remained fairly constant at 20° C. This constancy remained in spite of a further decrease in aortic pressure. It would seem advantageous, therefore, to lower the temperature rapidly, especially during the early phase of hypothermia when the coronary blood flow is relatively reduced. Lewis¹⁸ stated that the only incidence of irreversible ventricular fibrillation which he has encountered was in a patient in whom rapid cooling was used. He, therefore, prefers relatively slow cooling.

2. *Rate of rewarming.* Lewis¹⁸ found a higher survival rate in rats which were rewarmed rapidly. Lind and Senning¹⁹ noted small necrotic foci in the myocardium of dogs in all of their hypothermic experiments and felt that time might be a factor; that is, the longer the duration of the experiment, the more extensive the lesions. Drift occurs with rewarming just as it does with cooling and one should discontinue active warming when the body temperature is within 2–4° C. of normal. This minimizes the development of hyperpyrexia.

3. *Optimal temperature.* This temperature depends on whether or not one is using hypothermia as an adjunct or whether one plans to interrupt the cardiac venous return for intracardiac surgery. When used as an adjunct, one desires to reduce the oxygen consumption significantly but, at the same time, not reduce the temperatures to levels which carry a greater risk of serious cardiac arrhythmias. At 30° C. the oxygen consumption is roughly one-half of normal and the general level of 29° to 32° C. is being used by most investigators when the entire circulation is not interrupted. When inflow occlusion is used, a lower temperature is desired. A range of 25° to 28° C. is generally advocated and Swan²¹ states that for periods of inflow occlusion as long as 12 minutes, the temperature should be lowered to at least 25° C. Below 30° to 32° C. the risk of hypothermia becomes greater because it is generally agreed that ventricular arrhythmias begin at approximately this level and become more frequent as the temperature is reduced. In achieving the optimum temperature, one must take into consideration the drift which occurs after surface cooling has been discontinued. The drift is usually one-half to two-thirds the number of degrees of lowering at the time the cooling agent is discontinued, but depends on a number of factors including the rate of cooling and the size of the patient. If cooling has been rapid, it is likely that the drift will be greater. Drift is likely to be greater in large patients and adults and less in the smaller patient where

the depth of tissues which must be transcended by the decreasing temperature wave is less. Blood stream cooling is more uniform and drift is minimal.

4. *Prevention of shivering.* Shivering should be prevented during the process. When it occurs, cooling is more difficult. The oxygen consumption during shivering is greatly increased. It is especially important, therefore, to prevent shivering in cyanotic patients where one desires to maintain as high an oxygen saturation in the circulating blood as possible. When shivering occurs, it may be controlled by administering small doses of the muscle relaxants and if the anesthesia is especially light at this point, one may deepen it.

5. *Degree of ventilation.* The majority of those questioned used hyperventilation during hypothermia. Its use is based on the work of Swan and associates¹ in order to maintain a relatively constant pH during the cooling process. Lewis and associates,¹⁸ however, use an artificial respirating system throughout in order to maintain a constant respiratory rate. CO₂ (5 per cent) is added to the respiratory mixture to avoid the effects of hyperventilation at low temperatures and prevent the development of respiratory alkalosis.

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PROBLEMS IN METHODS OF INDUCING HYPOTHERMIA BY USE OF DRUGS AND INTERNAL COOLING

J. FRANCIS DAMMANN AND WILLIAM H. MULLER, JR.

External cooling by means of ice or cold water immersion has been the method selected by the majority of surgical teams¹⁻²¹ in this hemisphere. Several of the European groups²²⁻²⁷ have believed, however, that problems and hazards involved in hypothermia have been decreased by the use of a direct blood cooling unit. Thus, investigation in this country has split along two lines: (1) The investigation of an extracorporeal pump-oxygenator system, and (2) the investigation of hypothermia by means of external cooling. The Europeans have combined these techniques and have, at least in part, demonstrated that the combination simplifies both hypothermia and the extracorporeal circulation.

Direct cooling of blood in an extracorporeal circuit was first introduced abroad by Boerema²² in 1951 and Delorme²⁴ in 1952. Their work was extended by Ross and Brock^{23, 26, 27} and blood stream cooling is now being used by Brock during intracardiac surgery on humans. In this country Gollan²⁸⁻³⁷ has been the chief exponent of direct blood stream cooling. His work has been in the experimental animal, and experience in human patients has been very limited.

There are three different methods of direct blood stream cooling. Following Delorme's work, Ross and Brock²³ have developed a technique of vein-to-vein cooling. Blood is sucked through a catheter from the superior vena cava and, after being pumped through a siliconized polyethylene cooling coil, is returned to the inferior vena cava. If the thoracic cage is not opened, catheters are inserted into the superior and inferior vena cavae through the external jugular and femoral veins. During thoracotomy it was found that the most efficient method was the insertion of both catheters into the right auricular appendage; one catheter being directed up the superior vena cava and the other down the inferior vena cava. When starting a cardiac operation, Brock is prepared to use hypothermia but the final decision is not made until the chest is opened and the cardiac malformation is analyzed. If cooling is elected, the catheters can be inserted rapidly and the blood cooled at a rate of one degree centigrade per five minutes. Brock feels that there are several advantages to this method of cooling: It is rapid, readily reversible, can be applied easily in the open chest, and does not involve cannulation of any major arteries.

The second method consists of withdrawing blood from one of the large systemic veins and, after passing the blood through a cooling coil, returning the blood to the patient through an intra-arterial catheter. This technique was advocated by Ross and Brock as a means of increasing coronary blood flow and thereby improving cardiac function. In the experimental animal a striking improvement in heart action and a reduced incidence of ventricular fibrillation during ventricular cardiomy was noted when a simple venous-arterial cooling circuit was added. Later, they modified this simple circuit by the addition of a reservoir of oxygenated blood so that the oxygen content of the perfusing venous blood was increased. Lewis and co-workers^{19, 20} also have perfused the coronaries with venous blood at low tempera-

tures and noted an improved tolerance to prolonged cardiac manipulation. This method of cooling has the advantage of increasing or controlling coronary blood flow and systemic blood pressure. The chief disadvantage is that one is introducing into the arterial system unoxygenated blood. Furthermore, if clots form in the cooling coil, they are introduced into the arterial circulation and may damage a critical area. Using the vein-to-vein method, any clot that forms in the cooling coil is filtered out by the pulmonary capillary bed and the systemic circulation is protected.

The third and perhaps most promising method involves vein-to-artery cooling plus some form of oxygenator. This method has been extensively studied by Gollan, using an oxygen-diffusion oxygenator. Peirce has used vein-to-artery cooling plus a dog lung as the artificial oxygenator.²⁸

All three methods are open to certain criticisms. Cannulation of veins alone or a vein plus an artery adds to the total trauma. A complicated pump and cooling coil that needs careful watching is added to an already complicated setup. When an artificial oxygenator is used, the machine becomes even more complex. Any mechanical device is subject to breakdown and if the breakdown occurs at the wrong moment, the result may be a fatality. When difficulties do occur, it is not always easy to determine whether the extracorporeal circulation or hypothermia is at fault. When blood is passed through a system of coils and through an artificial oxygenator, damage may be done to some of the constituents of normal blood. Blood clots, hemolysis, loss of platelets and alteration in electrolytes comprise a few such alterations. These comprise the chief disadvantages of blood stream cooling.

In the questionnaire to which Dr. Muller referred, rapid cooling was preferred by the majority of those queried. If this view is correct, blood stream cooling would seem to offer a distinct advantage over other methods. The rates of both cooling and rewarming can be greatly accelerated. The total period under anesthesia is decreased. The surgical procedure can be started immediately and by the time the chest is entered, the patient's temperature will have reached the desired level. At low temperatures where dangerous arrhythmias seem more common, heart action can be observed directly and complications treated rapidly. As soon as the procedure within the heart has been completed, rewarming can be started. Consequently, the patient's temperature can be brought up above the danger level before the chest is completely closed. Swan⁸ has stressed the importance of warming to the point where pulse and pressure are strong and regular before the chest is closed, for he feels that complications can be recognized more rapidly and handled more efficiently with the chest open. Brock,²³ using vein-to-vein venous warming, has found that 20 to 30 minutes rewarming can be provided while the chest is being closed and that during this time the temperature can be raised to 32° C.

A common experience when external cooling of the body is used is the drift of the temperature downward after external cold is removed. Such a drift is not associated with blood stream cooling. The desired temperature can be selected, reached, and maintained until the procedure is completed. If, for reasons of necessity, rapid rewarming is desired, this can be carried out within a few minutes.

Ross states that a fall of one degree centigrade in five minutes is ideal when using the vein-to-vein method. Cooling can be faster but he does not feel that rapid cooling is as well tolerated by the animal. One result of very rapid cooling is that the

rate of body cooling is not uniform. The right auricle and right ventricle are cooled more rapidly than the rest of the body. Consequently, the SA node, which is highly susceptible to cold, is depressed. Cardiac output is decreased. Ventricular arrhythmia is more likely to appear. This objection would not appear to be valid when a venous arterial method of cooling is used. Gollan^{30, 31, 37} has cooled dogs down to five degrees in 40 minutes; and Peirce,³⁸ to twenty degrees centigrade in 20 minutes without ill effects. If the arterial cannula is inserted high in the arch of the aorta, cooling is essentially uniform and can be carried out rapidly. If the cannula is inserted into the femoral artery, cooling proceeds more rapidly in the lower extremities. By careful localization of the arterial cannula, therefore, either uniform or selective cooling can be carried out. As Peirce has suggested, selective cooling of the heart and brain may be the ideal approach.

All studies of hypothermia have shown a rise of three or four hundred per cent in oxygen consumption when the animal is shivering. Since one of the primary aims of hypothermia is to decrease the metabolic rate, the avoidance of shivering would appear to be a prime requisite of any method of cooling. Shivering has been avoided by: (1) using deep anesthesia, (2) adding to a mild anesthesia one of the curare drugs, and (3) using Thorazine and Phenergan, the French lytic cocktail.^{16, 40-45} Jung and his associates⁴⁶ established that shivering stimuli arise from receptors in cold skin. External cooling applies the cooling agent directly to the skin and, thus, a strong stimulus to shivering is promoted. Blood stream cooling avoids this reflex and shivering is decreased. As stated by Ross,²⁷ "By direct cooling of hypothalamus, adrenals and thyroid, this method aims at circumventing the highly efficient and coordinating reactions of the body to the surface application of cold." Furthermore, Ross has pointed out that shivering can be avoided completely if the skin is kept relatively warm. Thus, blood stream cooling would seem to offer a safer means of controlling shivering than the use of high doses of anesthetic agent or curare. In contrast to external cooling, there is no definite evidence of injury to nerves, fat or skin from blood stream cooling. Such injury might be difficult to detect. Damage to the constituents of blood, however, is more frequent with a simple extracorporeal circulation than when hypothermia is added.

Perhaps the most important advantage of vein-to-artery blood stream cooling is that at low temperatures it permits easier control of coronary perfusion rate, systemic pressure, and systemic blood flow. If the patient does develop a dangerous complication, it is possible to maintain life by maintaining an adequate coronary and cerebral blood flow until the complication is corrected. Brock is not concerned when ventricular fibrillation develops during cardiac occlusion. He feels that fibrillation may even be advantageous from the point of view of avoiding air embolisms and providing a quiet heart. Senning,⁴⁷ Juvenelle, Lind²⁵ and Wegelius have induced fibrillation to achieve a quiescent heart. Crafoord⁴⁸ found it difficult to maintain deliberately induced ventricular fibrillation while using a pump oxygenator. Brock permits the ventricular fibrillation to continue until the procedure on the heart has been completed and then defibrillates the heart with electrical shock following a period of cardiac massage and intracardiac adrenalin. He makes no effort to warm the patient until defibrillation has been accomplished and may continue decreasing body temperature if ventricular fibrillation appears before the patient has reached a sufficiently low temperature.

Hypothermia is alleged to predispose to the development of ventricular fibrillation. Many authors have concluded that it is not wise to lower temperature below twenty-six degrees centigrade because of the high incidence of ventricular arrhythmia. For the same reason, some authors have felt that hypothermia was indicated only in intra-auricular or great vessel surgery but not in surgery involving entering the ventricle itself. Lillehei¹⁹ has repeatedly stated that it is mandatory to maintain normal temperatures in patients undergoing a ventriculotomy during cross-circulation.

There is some reason to doubt that hypothermia, itself, increases the tendency toward ventricular fibrillation. Lewis's work with carbon dioxide,^{18, 20} Osborn's²⁰ studies of pH changes during hypothermia and a recent publication of Covino and Hegnauer²¹ all indicate that changes in blood pH are the important factors in the production of ventricular fibrillation rather than hypothermia. Hegnauer demonstrated that alterations in pH in both directions increased myocardial irritability. In the experimental animal when pH was controlled, hypothermia had little direct effect on myocardial irritability. All authors have agreed that pH tends to shift towards the acid side, particularly at a temperature of twenty-six or below. Osborn suggests that this shift is related to a total reduction in pulmonary blood flow, for although the blood carbon dioxide level is high, the alveolar carbon dioxide level is low. Vigorous hyperventilation is helpful but perhaps is not sufficient. Occlusion of the heart during an intracardiac procedure certainly alters pH. During the period of complete occlusion, circulation ceases, carbon dioxide accumulates and, immediately after the resumption of circulation, there is a marked increase in blood carbon dioxide. Swan attempts to control this shift by vigorous hyperventilation before the period of occlusion. He makes every effort to have the venous pH level above 7.5 immediately prior to occlusion.⁸ Osborn increased the total available base by submitting animals to a prolonged high carbon dioxide environment prior to cooling, believing that the acidosis developing at very low temperatures could then be buffered more readily.

It is not the purpose of this discussion to consider alterations in electrolytes, alterations in pH or the possible causes of ventricular fibrillation during hypothermia. It is important to point out, however, that no matter what the mechanism of pH shift, vein-to-artery cooling combined with an artificial oxygenator offers an excellent method of maintaining a normal blood pH. Furthermore, no matter whether ventricular fibrillation is brought about by change in blood pH, by reduction of coronary perfusion,⁵² by decreased efficiency of the myocardium⁵³ or by the type of anesthetic agent,⁵⁴ the use of the venous-arterial cooling system combined with an oxygenator would appear to offer great promise in reducing the high instance of ventricular fibrillation at low temperatures. Gollan⁵⁵ has been consistently able to lower the temperatures of experimental animals to zero to four degrees centigrade, producing cardiac arrest and not ventricular fibrillation. The rate of resuscitation has been excellent (13 out of 13). The incidence of post-hypothermic shock was zero in contrast to the high incidence reported by Wegelius and Gollan when the extracorporeal circulation was used only after cooling had been accomplished. Although he suggests that a continuous quinidine drip prevents ventricular fibrillation, it is hard not to implicate the pump-oxygenator rather than quinidine.

In animals which were cooled by refrigerated air and ice immersion, Gollan was not able to reach as low a temperature and the percentage of long-term survivals was markedly decreased.

Some of the problems involved in cooling and rewarming can be emphasized by citing a personal experience. Using the surface application of ice, we lowered the temperature of a newborn infant with a transposition of the great vessels to the surprisingly low level of 11 degrees centigrade. The heart did not fibrillate but went into standstill. At the end of the procedure, an effort was made to warm the baby: hot water bottles were placed around the infant and warm saline was used on the heart and the chest: cardiac massage was instituted to restore some degree of circulation. The temperature was raised to 26° C. degrees at the end of an hour and a half. At around 20° C., heart action returned, though the ventricular contractions were not sufficient to maintain an adequate pressure. At 26° C., heart action appeared to be improving but then became progressively worse. Cardiac massage was carried out for a two-hour period during this rewarming. Massage was necessary, for the only way in which warmth could be spread throughout the body was by producing a circulation. On the other hand, the only method of obtaining an adequate circulation was to have an adequate heart beat which in turn was dependent upon sufficient rewarming. It was our feeling that perhaps the chief cause of the death of this child was prolonged cardiac massage. At the start of cardiac massage, the appearance of the myocardium was excellent; but, as massage continued, small petechial hemorrhages appeared and the myocardium gradually became increasingly edematous. Had we had a vein-to-artery warming system at hand, it seems probable that we could have warmed with more speed, maintained coronary circulation without massage and, thus, avoided the trauma to the myocardium. If the desired aim is to lower body temperature to where cardiac standstill appears and where the circulation can be cut off for a prolonged period of time, it appears to us that we cannot rely on external methods of warming but must be able to maintain a circulation artificially until such a time as the heart is able to resume normal function. It may be that the temperature of 26° to 28° C. that has been termed ideal by many authors is not ideal. It is possible that a much lower temperature would offer more safety, besides permitting a longer period of circulatory arrest without damage to the heart and brain. If external methods of warming are used, the risk of going to such low temperatures is certainly great; for to rewarm, one must have a circulation and to have a circulation, one must rewarm. The use of a venous-arterial cooling and warming system may eliminate this problem and permit much lower temperatures to be reached with less danger and better results.

During the past few years a great deal of interest, time and effort have gone into development of an adequate artificial oxygenator and pump, one that can maintain life while the inflow and outflow tracts of the heart are closed. The difficulties of such a machine are tremendous; it must be efficient, safe, capable of handling large volumes of blood and not productive of damage to various components of blood. Many of the difficulties involved in the production of an adequate pump and oxygenator can be answered satisfactorily by the addition of hypothermia. At low temperatures, life can be sustained with a much smaller systemic blood flow. Consequently, a pump-oxygenator used with hypothermia would be required to handle a

much smaller volume of blood. This makes a smaller, cheaper and more efficient machine possible. At low temperatures, the normal clotting mechanism is interfered with. Consequently, when the pump is in use, a much lower heparin dosage can be used. Hemolysis of blood in the tubing of the pump is reduced by the reduction of temperatures. A reduction in blood flow means a reduction in coronary circulation and therefore a reduction in the amount of blood that enters the operative field from the coronary sinus. Finally, a pump-oxygenator is a man-made machine. It is, therefore, subject to breakdown. If a breakdown does occur when the temperature of the patient is low, the low metabolic rate permits more time for repair.

The final method of cooling which we will discuss involves the use of the French lytic cocktail,^{43, 44} either alone or in combination with external cooling. The basic ingredients of the French lytic cocktail are Thorazine and Phenergan. Both drugs belong to the generic group phenothiazine. Thorazine is primarily a central nervous system depressant. It is a mild antispasmodic, antihistaminic and adrenolytic drug. It is alleged to potentiate hypnotics, sedatives, narcotics, anesthetics, alcohol and antispasmodics. It may potentiate carbon dioxide narcosis. It produces sedation, probably by interrupting impulses passing between the diencephalon and cerebral cortex. It is not a hypnotic and does not produce addiction. Its value in hypothermia is that it blocks the thermo-regulating mechanism, thus permitting a closer approximation of body temperature to environmental temperature. Phenergan is a strong antihistaminic but a poor antithermic. One of its chief effects is decreasing capillary permeability. When these two drugs are used in combination with a small dose of Demerol, a state of drowsiness is produced. The normal body response to the application of ice is greatly inhibited. The peripheral vasoconstriction constituting the body's first line of defense against cold is markedly reduced and shivering, the second line of defense, is minimized. Thus, the patient can be cooled more rapidly. In experimental work, Dundee⁴² found that shivering could be controlled equally well with deep anesthesia, curare, or Thorazine. Angus Smith⁴⁰ used the lytic cocktail without additional external cooling and noted a drop in temperature to 95° F. In America, Ripstein¹⁶ has combined the use of Thorazine with a cooling blanket. Temperature is reduced to 83 in about an hour and a half. The patient is first anesthetized and placed on a refrigeration blanket. A 50-mg. dose of Thorazine is given intravenously, followed by 100 mg. intramuscularly. Then, 50 mg. are given every one to two hours during the procedure and for rewarming. Dr. Ripstein feels that the chief advantages of this technique are a more rapid fall in temperature and the avoidance of shivering without the use of curare or a heavy anesthetic.

It is difficult to assess the advantages and disadvantages of the French lytic cocktail. Most workers in this country have avoided use of the cocktail because Thorazine and Phenergan are potent drugs with diverse pharmacologic effects. The dosage is variable. The degree to which hypnotics and barbiturates are potentiated varies. Since the hazards of hypothermia still have not been thoroughly elucidated and controlled, it seems unwise to add to the difficulties potent drugs, the actions of which are not clearly understood.

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DISCUSSION

Dr. J. Adams-Ray: A new method for producing hypothermia has been elaborated in team work with engineer P. O. Persson at the Royal Institute of Technology, Stockholm.¹ Cold air with a velocity of 5–6 meters per second is fanned over the dog or patient, lowering the rectal temperature by 0.1° C. per minute, much as is done with icewater. Heating elements provide warm air for the rewarming period.

As we all know, it may be difficult to keep the rectal temperature stable, and drift may be dangerous. When studying the effect of hypothermia on burns with Dr. B. Johansson and L. Troell we worked out a technique for the automatic control of the rectal temperature. A thermocouple adjusted for the desired rectal temperature is inserted in the rectum. When the temperature has come down to 31° C., the automatic-control thermocouple is activated. The rectal temperature can be held at a constant level very easily (fig. 1), in this case a 24-hour experiment. The oscillation of the air-temperature can be adjusted by a maximum-minimum control (see the adjustment at 12 hours).

An apparatus built on these principles and that can be used for experiments or as a cooling bed or operating table is now manufactured by the Heljestrand Cy. Eskilstuna, Sweden (fig. 2). The air temperature can be varied from -10° C. to

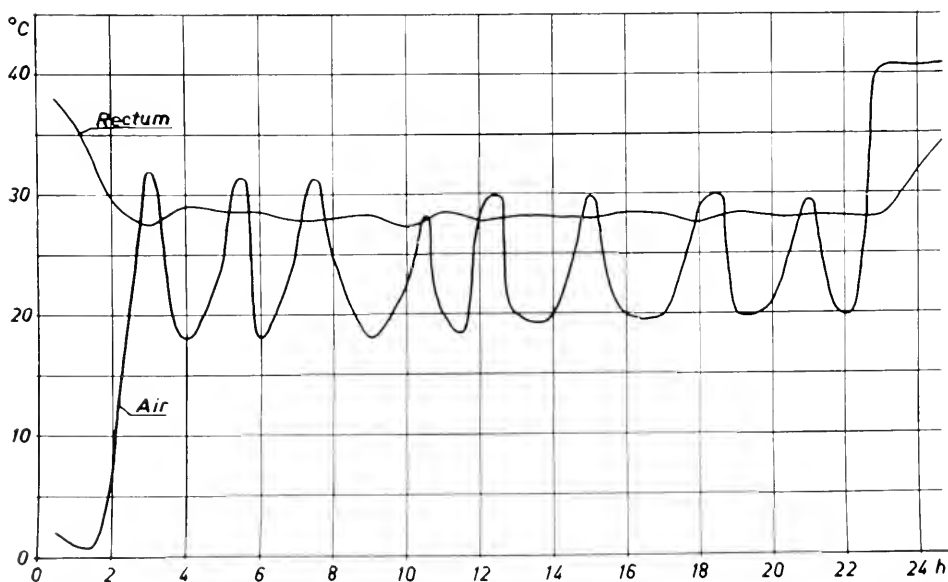


FIG. 1.—Maintenance of constant rectal temperature by automatic control of air temperature.

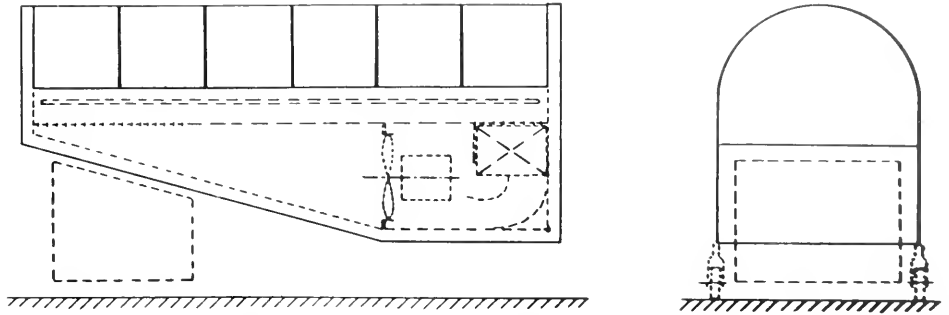


FIG. 2.—Schematic drawing of apparatus. To the right are located the fan, the cooling coils, and the heating elements. Under the left side of the apparatus is located the refrigeration system.

+50° C. The segmental plastic domes can be removed and the operation-field draped off (fig. 3). A special apparatus for neuro-surgical operations and one with possibility of tilting the patient and adjusting the height of the apparatus has been constructed. The patient can thus be brought to the wanted rectal temperature, kept there automatically and warmed in the same apparatus in an easy, simple, and neat manner.

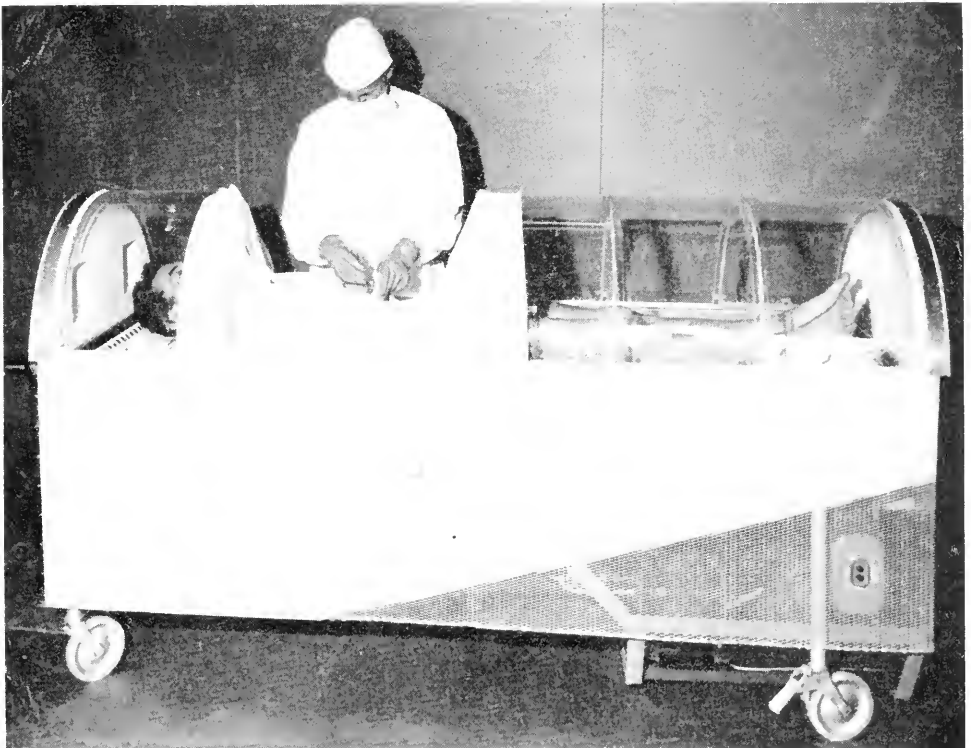


FIG. 3.—Apparatus in use.

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Dr. A. Riberi: We have had a good deal of experience with experimental hypothermia, and have used a bath of water and crushed ice. We have never had any trouble with our dogs during cooling. We have never had any case of ventricular fibrillation due to hypothermia alone in about 400 dogs operated up to the present time. We also use a cooling blanket when we operate on adults. With children we have used ice bags; this is a very simple technique and, so far, we have not had any trouble with fat necrosis. In this way hypothermia is induced in children very fast. It takes about 15 or 20 minutes to cool a small child from 37 down to 30–28 degrees centigrade, at which time the patient is put on the blanket, which is rewarmed as soon as the cardiac part of the operation is over. When the blanket is used for cooling, the time required is long and the surgeon is obliged to wait, sometimes hours, before the patient has reached the temperature desired. This can be obviated by careful planning.

I wish to emphasize Dr. Muller's point that the blanket is very helpful, but also that the danger of burns is a real one and must not be overlooked.

Dr. R. O. Heimbecker: Many factors help to determine the pHi changes and value of assisted ventilation under hypothermia. The method of cooling and degree of anesthesia are important.

We find that surface cooling with blankets is quite a different situation from cooling with ice water, in that the latter is a strong stimulus to spontaneous hyperventilation by the anesthetized patient.

In cardiac cases in which the chest is, of course, open, hyperventilation is important, while in the neurosurgical cases in which the chest is closed, spontaneous respiration seems adequate.

Dr. F. Gollan: The induction of hypothermia by means of a small pump-oxygenator has drawbacks as well as advantages. The drawbacks are due to difficulties in the technique of extracorporeal circulation and oxygenation. They can be overcome by systematic experimentation under controlled conditions. The advantages are of a physiological nature, and I am confident that those investigators who are patient enough to master the technique will be gratified by the results.

This is not the place to go into a detailed description of the instrument and I just would like to show a diagram of the principle used (fig. 1). Oxygen is forced through a microporous porcelain disc into venous blood which is promptly oxygenated by the dispersion of these fine bubbles (Clark, Gollan and Gupta in 1950). The fine oxygen bubbles are then made to coalesce into large ones by leading the blood over a large surface coated with an antifoam compound. The large bubbles are separated from the blood by a screen and rise to the lower surface of another porcelain filter of much larger pore size. This filter is made non-wettable so that the blood will be repelled whereas the excess of free oxygen and carbon dioxide can filter through. Thus, by a change of surface tension a small closed and disposable plastic chamber can oxygenate 2 liters of blood per minute. Circulation is provided by a finger-pump and temperature changes are produced by a coil in the system.

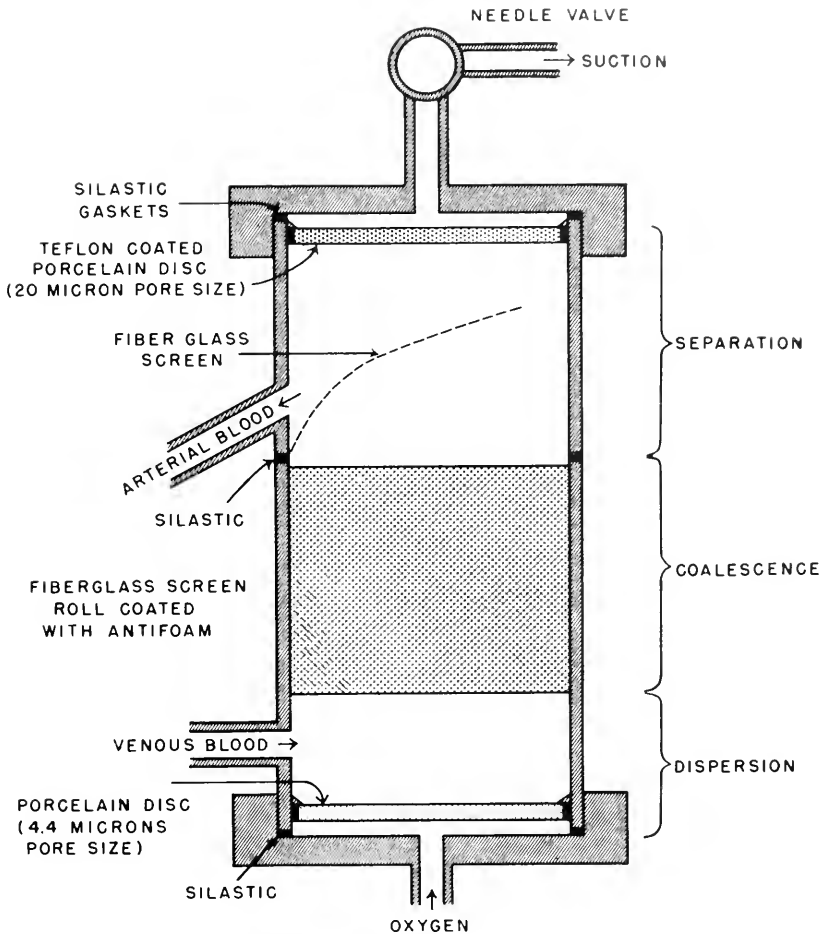


FIG. 1.—Diagram of a small pump-oxygenator.

By immersion of the coil in an ice-salt mixture, differential hypothermia (fig. 2) can be rapidly induced in the upper part of the dog's body where the temperature can be lowered to or close to 0°C . This might not be necessary in eventual clinical applications. We did it only to prove that the temperature at which water freezes is not harmful to large, adult, nonhibernating mammals.

At about 13°C , the last heart beat is recorded (fig. 3) and cardiac arrest can be maintained as long as the temperature is kept that low. The heart is still irritable because mechanical stimulation can elicit a slow contraction at 6°C . On rewarming the blood the heart starts to beat again in sinus rhythm and at about 30°C , the pump-oxygenator can be turned off.

This technique has the following advantages: it is rapid and clean; hypothermia is selective for organs perfused with cold blood; no drifting to lower temperatures takes place; shivering is minimal or absent; the volume of the instrument is so small that it can be filled with cold Ringer's solution; the increased oxygen consumption of rewarmed organs is simultaneously and automatically covered with an adequate

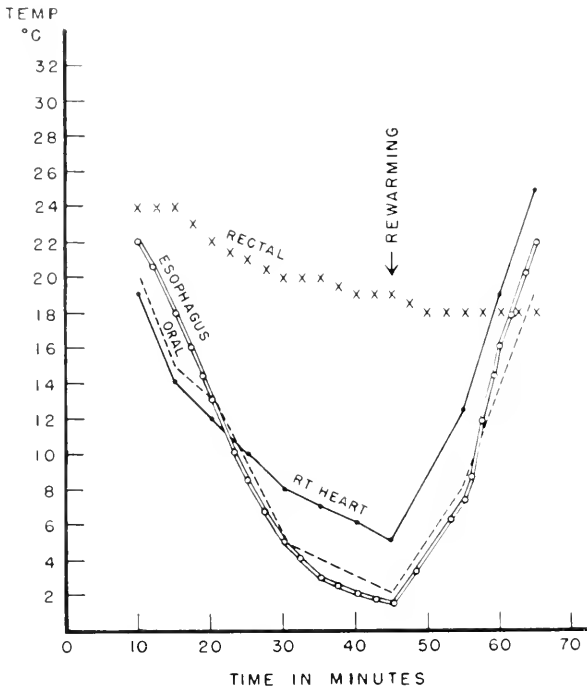


FIG. 2.—Selective body hypothermia during blood cooling.

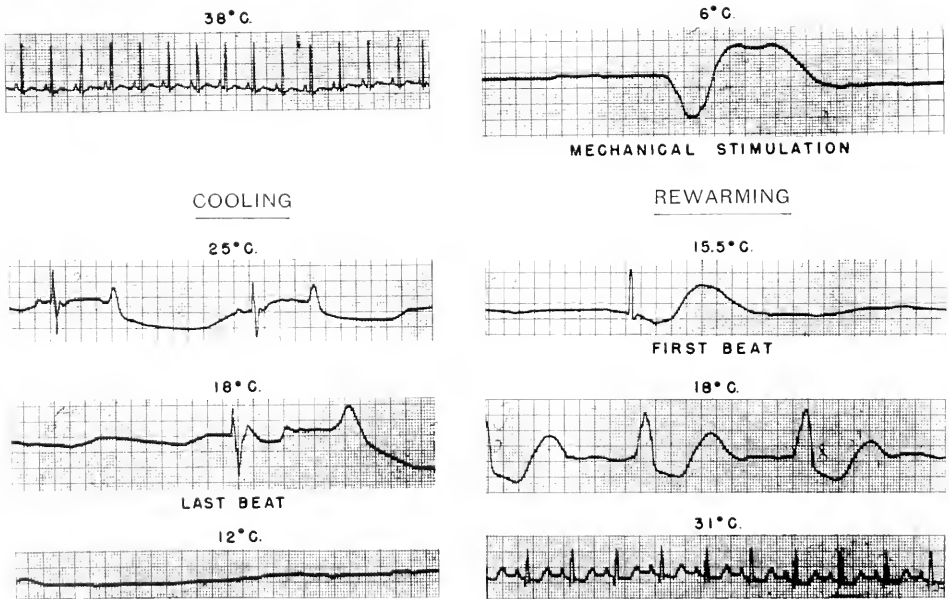


FIG. 3.—Electrocardiograms in severe hypothermia by blood cooling.

supply of oxygenated blood; a small amount of heparin is sufficient to keep the blood liquid at low temperatures; hemolysis is minimal; the cannulation of peripheral blood vessels only supplies adequate blood flow of about 10 to 20 ml/kg/minute and trauma to the large intrathoracic vessels is avoided; the operating field is not crowded by tubes; the collapsed lungs and the heart in arrest permit the leisure and accuracy essential for the surgical repair or replacement of delicate anatomical structures; low blood flow and aortic pressure reduce the coronary flow to a mere trickle; ventricular fibrillation is absent in quinidine pretreated animals; air embolism does not occur since the heart in asystole does not eject blood and air into the aorta; tissue anoxia is prevented during rapid cooling and rewarming; the time for open cardiomyotomies is not limited to minutes, but to hours.

Dr. R. W. Brauer: I should like to call your attention to yet another method of cooling devised by Drs. F. W. Behmann and E. Bontke in Prof. Thauer's laboratory at the Kerckhoff Institut, Bad Nauheim, Germany. In this procedure a plastic catheter is introduced into the vascular system in such fashion that cooled brine can be circulated through it, resulting in intravascular cooling of the blood. The general arrangement is shown in figure 1. The catheter K is introduced into the femoral vein and is guided under a fluoroscope past the heart to an exit point in the jugular vein by means of a special auxiliary catheter with a pickup device. The temperature of the circulating brine is regulated in passage through the thermostat bath Th, equipped with heating and cooling systems. These are controlled by the relay R activated by the rectal resistance thermometer W, and by the maximum-minimum control Gr. The system allows rapid lowering of body temperature of lightly

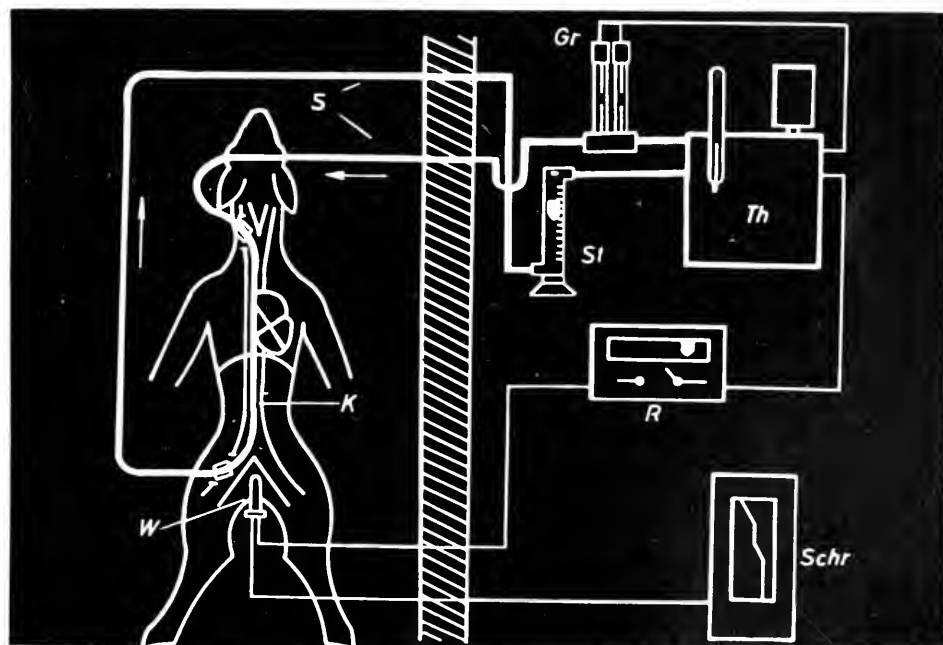


FIG. 1.—Diagram of Kerckhoff Institut hypothermia preparation using intravascular brine cooling of blood.

anesthetized dogs, and maintaining it quite steadily at rectal temperatures down to 25° C. at least. The maximal cooling efficiency is conditioned by the dimensions of the catheter in relation to the large vessel diameters to a greater extent than by the rate of blood flow, at least within the range studied so far.

The advantages of this technique are the relatively atraumatic procedure resulting in minimal derangement of normal circulatory conditions, the possibility of maintaining a steady predetermined degree of hypothermia while maintaining any desired room temperature, and the possibility of continuously measuring and recording the number of calories withdrawn or added in relation to the animal's temperature pattern. Limitations of the procedure are that the maximal rate of heat withdrawal cannot well exceed a value of 1200 cal./min. \times m², and the obvious dependence of cooling upon a functioning circulation.

In all, this technique of intravascular cooling would seem to have a great deal to offer in the study of temperature regulation as well as of the physiology of the relatively undisturbed hypothermic animal; although no clinical applications have been attempted so far, one can envision a number of situations where the close temperature control effected by intravascular cooling could be of very real value.

Dr. L. I. Goldberg: In a study of the effects of total, preganglionic, sympathetic block (produced by epidural injection of 0.45 per cent procaine solution) on cardiac arrhythmias developing during hypothermia in the dog, it was found that ventricular fibrillation occurred at higher temperatures in blocked animals than in dogs without such sympathetic block. Hypothermia was rapidly produced, in the initial series, by a veno-venous, extracorporeal method, in which blood was circulated through 8 feet of plastic tubing immersed in water at 0° C. In a later, smaller series with blocked dogs, experimental conditions were unchanged, except that hypothermia was produced more gradually by application of ice packs. The animals, cooled in this manner, developed ventricular fibrillation at significantly lower temperatures than the animals cooled rapidly by the extracorporeal shunt. In a third series of blocked dogs, hypothermia was produced by the extracorporeal shunt, but the rate of temperature decrement was controlled in order to approximate the more gradual rate of cooling produced by application of ice packs. Ventricular tachycardia or ventricular fibrillation developed at higher temperatures in three or four of these latter dogs, also, than in those made hypothermic by application of ice packs. Preliminary results are tabulated below.

Adult, mongrel dogs, weighing between 10 and 20 kg., were used in this study. Anesthesia was induced with intravenous thiopental sodium and maintained in the third stage of surgical anesthesia. Epidural, preganglionic, sympathetic block was produced according to the technique described by Brewster, Isaacs and Waino-Andersen (*Am. J. Physiol.* 175: 399, 1953). In this technique, laminectomy is performed and polyethylene catheters are inserted into the epidural space at varying levels. Procaine solution (0.45 per cent) is injected every 15 minutes through the catheters until sympathetic block is achieved. After a total block is established, additional procaine is injected every 30 minutes for the duration of the experiment. Non-blocked dogs were prepared in the same way, but normal saline solution was substituted for the procaine solution.

All dogs were mechanically ventilated by an Emerson Resuscitator with 100 per

cent oxygen through a cannula tied into the trachea. Mechanical ventilation was begun at least 30 minutes before the animals were cooled. The respirator rate was 15 per minute in Experiments 1, 2, 3, 5, 7 and 8. In all other experiments the respirator rate was maintained at 30 per minute.

Extracorporeal cooling was accomplished by means of a modified Dale-Schuster pump. Blood was pumped from the inferior vena cava via one femoral vein through 8 feet of plastic tubing immersed in cold water and was then returned to the animal via the other femoral vein. Approximately 150 cc. of dextran solution was needed to prime the pump. Electrocardiograms (leads I and II or I and III) and femoral arterial pressures were frequently recorded. Recordings were made synchronously on three channels of a Sanborne Poly-Viso oscillograph. Arterial pressures were obtained from a femoral artery by means of a Sanborne electromanometer. All temperatures were obtained by means of a mercury thermometer inserted approximately 20 cm. into the rectosigmoid.

In most cases, cooling was continued until ventricular fibrillation or cardiac arrest occurred. In a few experiments, rewarming was carried out by replacing the cold water surrounding the plastic tubing with water of 35–40° C.

The following are typical protocols:

SERIES I.—BLOCKED DOGS, RAPID, EXTRACORPOREAL COOLING

Expt. no.	Ventricular tachycardia (° C.)	Lowest recorded temp. (° C.)	Rate of cooling decrement per 15 min. (° C.)
1.....	31.7	29.5, VF	5.6
2.....	33.5	27.0, VF	3.0
3.....	—	24.5, VF	3.0
9.....	27.1	22.5, VF	5.0
12.....	26.3	16.0, CA	2.3
13.....	24.0	23.7, VF	3.8
14.....	29.4	22.4, VF	4.1
20.....	26.5	25.5, VF	2.8
22.....	29.5	24.0, VF	2.6

SERIES II.—NON-BLOCKED DOGS, RAPID, EXTRACORPOREAL COOLING

Expt. no.	Ventricular tachycardia (° C.)	Lowest recorded temp. (° C.)	Rate of cooling decrement per 15 min. (° C.)
5.....	20.7	19.5, VF	2.7
7.....	25.9	19.0, VF	3.5
8.....	—	17.0, RW	3.5
10.....	—	15.8, RW	3.7
16.....	—	19.0, VF	2.8
17.....	—	19.5, VF	4.1

Continued—next page

PHYSIOLOGY OF INDUCED HYPOTHERMIA

Continued—from preceding page

SERIES III.—BLOCKED DOGS, SLOW, SURFACE COOLING

Expt. no.	Ventricular tachycardia (° C.)	Lowest recorded temp. (° C.)	Rate of cooling decrement per 15 min. (° C.)
30.....	—	18.2, CA	1.1
31.....	—	20.5, VF	1.0
40.....	—	21.6, VF	1.7
41.....	21.7	18.9, VF	0.9
42.....	—	21.5, VF	0.9

SERIES IV.—BLOCKED DOGS, SLOW, EXTRACORPOREAL COOLING

Expt. no.	Ventricular tachycardia (° C.)	Lowest recorded temp. (° C.)	Rate of cooling decrement per 15 min. (° C.)
43.....	20.7	20.5, RW	0.9
44.....	—	24.3, VF	0.9
45.....	—	18.6, CA	1.0
46.....	31.3	20.2, RW	0.9

Abbreviations: VF, Ventricular Fibrillation; CA, Cardiac Arrest; RW, Rewarmed.

REVIEW AND APPRAISAL OF PART V

R. D. DRIPPS

Three methods of inducing hypothermia in man have received the most attention, i.e., (a) use of drugs alone, (b) application of cold to various body surfaces, and (c) cooling of blood removed from and subsequently returned to the body. Unfortunately, few comparative studies of these techniques have been reported in humans so that much remains to be learned about the optimum approach to reduction of body temperature in clinical practice. In this brief review established data will be presented, together with suggestions for future investigation.

It is evident that no single drug or group of drugs has produced a significant lowering of body temperature without added cold. Neither chlorpromazine, Phenergan, Hydergine, or meperidine, alone or in combination, have caused significant hypothermia. A few degrees reduction in body temperature over a two to four hour period is the most that can be expected in the average adult patient. Some or all of these substances are of value as adjuncts to cooling, but none can stand as a primary agent.

Direct cooling of blood appears to produce a more rapid reduction of body temperature in adults than does any method of surface cooling. Direct blood cooling is also alleged to be associated with less compensatory shivering and vasoconstriction. Damage to skin, subcutaneous tissue and peripheral nerves are infrequent with this method in comparison with surface cooling.

Unsolved problems include some of the following:

Ideal rate of cooling. From the practical standpoint the more rapidly one can lower body temperature, the less time is wasted. In infants and children temperature reduction can be achieved with extraordinary rapidity by surface cooling. This is not true in adults where greater surface area and increased subcutaneous fat slow up temperature change. The hazards of rapid cooling deserve further study. Irregularities in cardiac rhythm have been alleged to follow rapid hypothermia. The concomitant effect of such factors as inadequate ventilation, hypotension, and shivering has not been assessed, so that controlled experiments are needed before one can determine the safest rate of reduction of body temperature.

If a relatively rapid rate of cooling is found safe, can the lowering of temperature following surface cooling be increased by tilting the subject, changing his position regularly, or by brushing or rubbing the skin vigorously? Clinical impressions suggest that these adjuncts deserve appraisal.

Post-cooling downward drift of body temperature. Once active cooling has been stopped, a continued decline of body temperature has been reported by a number of workers. Again this appears more frequently in infants and children. The predictability of the extent of this drift is not reliable. The causes and prevention of this continued fall in temperature require investigation.

Anesthetic management. There is little agreement as to the pre-hypothermia sedation, the anesthetic agents and techniques, or the muscle relaxants which are preferable as cooling is induced. Substances which are destroyed in the body will

tend to accumulate as body temperature falls, since detoxification mechanisms are depressed. Animal studies suggest that ventricular fibrillation is less common with one type of anesthesia than another. This has not been established for man. General anesthesia and the administration of a muscle relaxant are generally used during surface cooling to minimize shivering and vasoconstriction. The ideal depth of anesthesia is unknown. Whether chlorpromazine, other phenothiazine derivatives, Arfonad, or various ganglionic blocking or adrenergic drugs should be part of the anesthetic regimen remains uncertain. Various beneficial effects including increased rate of cooling, protection against ventricular fibrillation and decreased cardiac work-load have been attributed to use of some of these substances, but controlled data are lacking.

Type of respiration. The majority of those reporting indicate a preference for hyperventilation techniques in an effort to achieve a mild degree of respiratory alkalosis. Disagreement with this has been voiced by a few. If hyperventilation is accomplished by increased pressure in the airway, a comparison of the hypotensive effects of intermittent positive pressure and alternating positive-negative pressures should be made.

Use of pumps and oxygenators. When direct cooling of blood is the method for producing hypothermia, one must consider the advisability of incorporating a pump and/or oxygenator in the circuit. Should well-maintained perfusion of the coronary vascular bed prove of value in reducing the incidence of ventricular fibrillation, the incorporation of such devices may be essential.

Rewarming. There is little agreement on the best method or on the optimal rate of rewarming a patient. The circulatory hazards during rewarming have been stressed by some and have proved of little significance to others. Overshoot of body temperature to above normal values probably represents the same sort of response reported as downward drift, only in the opposite direction.

Apparatus. Attention should be directed towards standardization and simplification of the technical details involved in hypothermia. Complex, costly apparatus should be avoided if possible. The ability to change body temperature while operation is in progress is also desirable.

Differential versus whole body cooling. If a single tissue such as the brain or liver is to be operated upon and interruption or reduction of the blood supply is necessary, localized cooling of the involved region would seem preferable to generalized body cooling. Various methods of producing differential cooling have been reported. The technical aspects should be simplified and refined.

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